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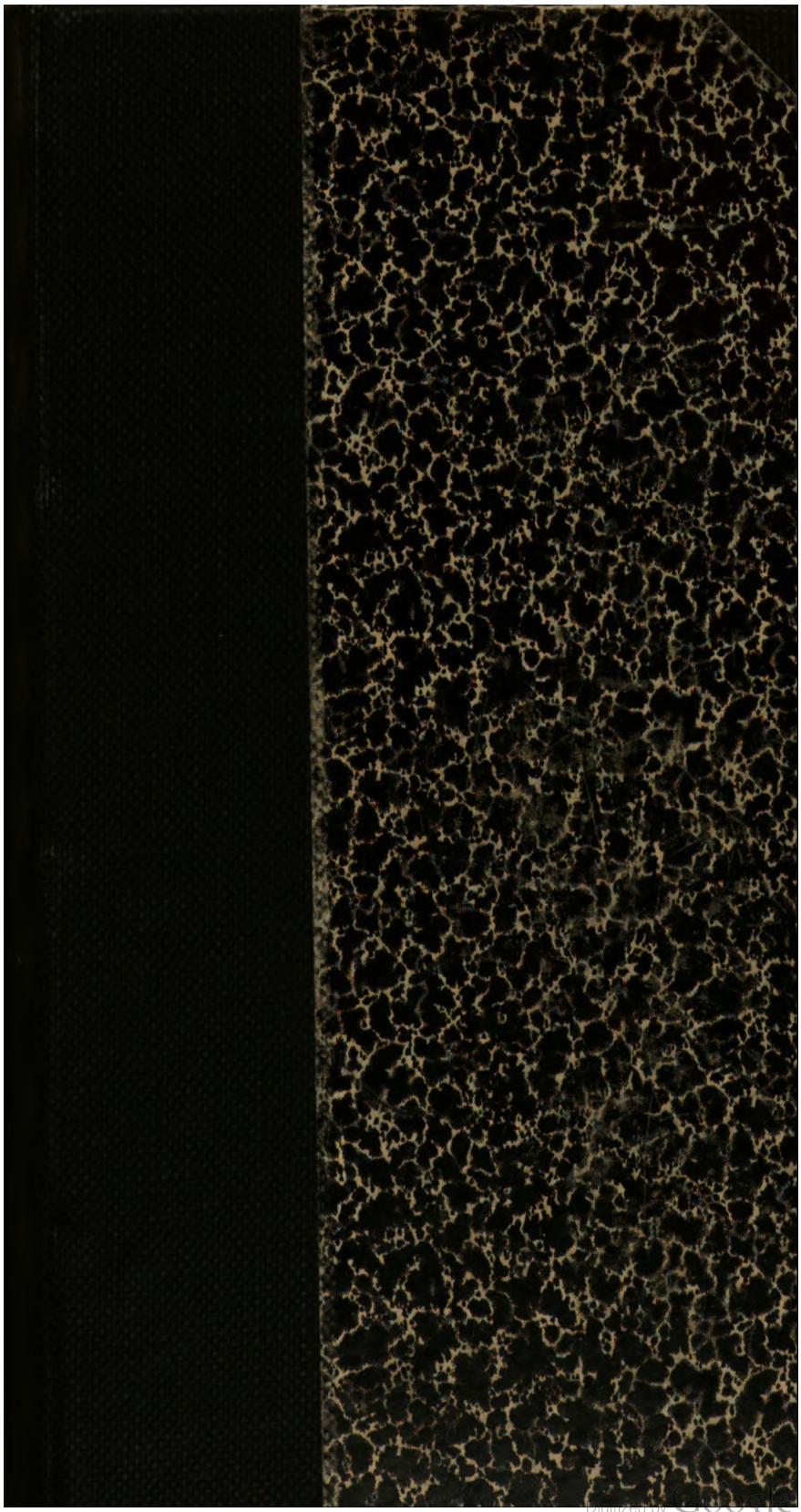
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ORGANIZED 1878 INCORPORATED 1891

JANUARY
1918

VOLUME XXXVII No. 1

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T. W. GALLOWAY,
Secretary-Editor.

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INSECTS AS CARRIERS OF DISEASE

By Malcolm Evan MacGregor
Wellcome Bureau of Scientific Research

So much has been said about insects since the War began that it is, I think, advisable that some attempt should be made to summarize our knowledge of the more important insect-borne diseases and their vectors. While insects have long been suspected of being responsible for the transmission of serious diseases, it may be said that practically the whole of our knowledge of insects in this rôle has been acquired within the past twenty years. So rapidly, however, has the charge of this offence been made out against them that, although it is common knowledge they have been proved guilty, it is not generally realized upon how many counts the verdict rests.

It has lately been my good fortune to give class instruction for the War Office to officers of the R. A. M. C. who are proceeding to the East, and, in order to bring home to my audience the importance of the connection between insects and disease, I have compiled the tables which I now publish. These can in no way claim to be complete, but merely present the more important insect-borne diseases, including important human diseases that on certain grounds are suspected of having insect vectors. With these tables I also publish one (Table VI) which includes the chief insects and acarina that are directly the cause of disease in man and his domestic animals. To complete the list of insect-transmitted diseases would demand the consideration not only of other mammals as hosts, but also of avian and reptilian hosts. In the present instance this would be to carry the subject beyond general interest, but it must be remembered, therefore, that, long as the present list of charges is, insects are not here arraigned on all the counts that might with justice be preferred against them.

Reprinted from Journal of Tropical Medicine and Hygiene, Sept. 15, 1917; xx, 18.

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During the last few years medical entomology has been rapidly establishing itself an an invaluable branch of preventive medicine, and with the outbreak of the present War a great deal of interest and study has been devoted to this subject in Europe, notably in connection with the transmission of typhus fever by lice, and the dissemination of bacteria and other organisms by flies. Moreover, the importance of insect vectors has been generally realized, and many of the astonishing interactions between pathogenic micro-organisms and certain arthropoda have become popular knowledge.

Centuries ago insects were suggested as being possibly concerned in the spread of disease, and from time to time such logical hypotheses were advanced that it is surprising that the establishment of the truth was not sooner forthcoming. In 1577 Mercurialis, an Italian physician, suggested that plague, which was then ravaging Europe, was spread by flies feeding upon the diseased and dead, and later depositing fæcal matter on food consumed by healthy persons. This idea held ground, and various suggestions occur as to the spread of disease by flies in the literature of the eighteenth century. Edward Bancroft in 1769 advanced the theory that "yaws" was transmitted by flies feeding on diseased subjects, and carrying the contagion by settling on open wounds or scratches on healthy persons.

In 1848 Dr. Josiah Nott, of Mobile, Alabama, published a remarkable article in which he gave reasons for supposing that yellow fever was an insect-borne disease. However, although he mentioned many insects, he did not specify any insect as the particular vector.

The connection between malaria and the mosquito had long been held, it is said, by the Italian and Tyrolese peasants, and even by the natives of East Africa, but the first charge brought against the mosquito in the spread of disease by scientific authority was in connection with yellow fever.

In 1853 Dr. Daniel Beauperthuy, a French physician, wrote ably arguing that yellow fever and other fevers were transmitted by mosquitoes, but in those days there being no accepted belief in a contagium vivum, he concluded that the virus was obtained from decomposing material that the mosquito had consumed, and which

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was later accidentally inoculated into man. Raimbert in 1869 showed by experiment that anthrax could be disseminated by flies.

Epoch-making in the history of our knowledge of insect vectors was Manson's discovery in 1878 that *Filaria bancrofti* was spread by mosquitoes, but at first he thought the *filariæ* escaped from the insect into water, and reached man in this manner. Later work by Manson and his colleagues determined the exact means of transmission.

It was not until twenty-eight years after Beauperthuy's theory that Charles Finlay, an American of Havana, in 1881 definitely attributed the transmission of yellow fever to a mosquito of a definite species. He had noticed the connection that seemed to exist between the presence of large numbers of *Stegomyia fasciata* and the prevalence of yellow fever. He then attempted to transmit the disease experimentally by the bites of this mosquito, and although his experiments are open to criticism, there is no doubt that he did succeed in doing so.

Three years later, in 1883, another American, A. F. A. King, advanced the first well formulated mosquito-malaria theory, and in 1898 Ross, in India, demonstrated beyond doubt the important rôle played by mosquitoes in the transmission of malaria.

In 1899 the American Yellow Fever Commission (Reed, Carroll, Lezear, and Agramonte) were sent to Cuba, and were there able to demonstrate with certainty that yellow fever is transmitted by *S. fasciata.*

It is interesting thus to note the almost parallel development in time of our knowledge of two of the most important insect-borne diseases. To deal even briefly with the historical aspects of our knowledge of other diseases tabulated below would be to consume a large amount of space, and the foregoing account will have indicated the path that has led to subsequent discoveries whose histories are readily available.

I will pass, therefore, to a few notes on each of the tables.

Notes to Table I.—Diseases of Unknown Origin

The majority of these diseases are doubtless caused by living viruses; often organisms of ultra-microscopic size, and commonly referred to as "filterable viruses."

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In the case of pellagra it would appear, however, from the most recent work that, although it is still considered by many persons to be a possible insect-borne disease (and, according to Sambon, having a likely vector in either the *Ceratopogoninæ* or *Simuliidæ*), Goldberger in America considers it a disease now certainly attributable to vitamine-starvation through an unbalanced diet. If this is the case, there is no causative organism and no vector, and pellagra should be ruled out of present consideration. The question, nevertheless, is by no means settled.

The virus of acute anterior poliomyelitis is still not isolated with certainty. Flexner and his colleagues have been able to cultivate a filterable micro-organism which produced the disease in experimental animals, and more recently Rosenow and his fellow workers have isolated a polymorphous streptococcus, with which they were also able to produce paralysis in certain animals. Nuzum and Herzog were able to do likewise by a Gram-positive micrococcus isolated from the brain and spinal cord of persons dead from the disease.

Poliomyelitis has been very generally suspected of being transmitted by insects, particularly by *Stomoxys calcitrans* (the stable-fly), fleas, and *Tabanidæ* (gad-flies). Nevertheless, it appears more likely that it has an ærial transmission, infection being acquired through the buccal and nasal mucous membranes.

The causative organism of Rocky Mountain spotted fever, Wolbach claims to have discovered in the bodies of infective ticks (*Dermacentor venustus*).

Notes to Table II.—Diseases of Bacterial Origin

In the majority of cases, diseases of this class have an *indirect* transmission by insects—that is to say, instead of the organism entering the body of the host through inoculation by the bite of an insect (*direct* transmission), the organisms are carried in or on the insect's body, and are deposited by contact on human food or skin abrasions, and in this manner cause infection.

Bacillus tuberculosis may be disseminated by house-flies feeding on infective sputum, as was first shown by Spillman and Haushalter (1887), and subsequently by the investigations of other workers.

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Table I.

The more important Insect-borne Disease of Unknown Origin

N. B.—Names between square brackets = certain vectors; names without square brackets = probable vectors; names followed by ? = possible vectors.

Organism	Host	Disease	Vector
?	Man ...	Dengue (breakbone fever) ...	[Sandflies (Phlebotomus), Mosquitoes. *C. fatigans; S. fasciata*]
?	" ...	Three-day fever, *syns.* "Dog disease," Sandfly fever, Phlebotomus fever	[Sandflies (Phlebotomus), Mosquitoes. *C. fatigans; S. fasciata*]
?	" ...	Yellow fever	[Mosquitoes *(Stegomyia fasciata)*]
?	" ...	Trench fever	Lice ?
? Salivary toxin? ...	" ...	Tick paralysis (American) ...	[Ticks, *(Dermacentor venustus)*]
? Salivary toxin? ...	" ...	Tick paralysis (Australian) ...	[Ticks *(Ixodes ricinus)*]
?	" ...	Rocky Mountain spotted fever	[Ticks *(Dermacentor venustus)*]
?	" ...	Japanese river fever (shima mushi)	[Mites *(Larval trombidiidæ)*] "aka mushi"
?	" ...	Acute anterior poliomyelitis ...	? Many insects have been claimed as vectors, notably *Stomoxys calcitrans?*
?	" ...	Pellagra	? Gnats of the genus Simulium have been claimed?
?	" ...	Typhus fever	[Lice]

Table II.

The more important Insect-borne Diseases of Bacterial Origin

N. B.—Names between square brackets = certain vectors; names without square brackets = probable vectors; names followed by ? = possible vectors.

The word "flies" includes in the main: *Musca domestica*, *Fannia* spe., *Calliphora* spe., *Lucilia* spe., and *Sarcophaga* spe.

Organism	Host	Disease	Vector
Bacillus anthracis	Man and animals	Anthrax	[Flies], *Tabanidæ? Beetles?*
" *dysenteriæ*	"	Bacillary dysentery ...	[Flies] *(Musca domestica, Calliphora* spe., *Lucilla* sps.)
" *lepræ*	"	Leprosy	Flies? Fleas? Bed-bugs? Skin mites? Mosquitoes?
" *paratyphosus A*	"	Paratyphoid fever ...	[Flies]
" " *B*	"	" " ...	[Flies]
" *pestis*	" and rats ...	Plague	[Fleas]
" *tuberculosis*	" and animals	Tuberculosis	Flies, cockroaches, fleas? Bed-bugs?
Bacillus typhosus	"	Typhoid fever ...	[Flies]
Bartonia bacilliformis x-bodies ...	"	Verruga	[*Phlebotomus verrucum*]
Spirillum choleræ	"	Cholera	[Flies], cockroaches, ants. Although the main channel of infection is the consumption of infected food and water

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With the high vitality and resistance to drying possessed by the *B. tuberculosis,* the possibly long incubation period within the body and the insidious onset of the disease, the danger from *Musca domestica* in this connection is still not sufficiently recognized.

Human infection with plague and typhus has been shown to be acquired principally by the entrance of the virus through skin lesions, the insect vector having been crushed either during or after the act of blood-sucking. The stomach contents or infected excreta may be rubbed into the lesions or gain entry through abrasions caused by scratching.

This, however, does not preclude the possibility of direct infection also occurring, at least sometimes in the case of plague, as the infected flea has the proventriculus occluded by the plague organisms when the flea infection is at its height. Septicæmia following mosquito bites occasionally happens, and as likely as not the pathogenic organisms are introduced when the mosquito bites. Direct transmission by blood-sucking insects may possibly also occur in certain instances in the spread of tuberculosis and leprosy.

If Wolbach's organism (see Table I and notes thereto) is proved to be the cause of Rocky Mountain spotted fever, this will also be a disease of bacterial origin with *direct* transmission through a tick, *Dermacentor venustus.*

Notes to Table III.—Diseases of Spirochætal Origin

With these diseases the usual method of transmission is *direct* —that is to say, through the bites of the insect vectors.

Exceptions occur in the case of relapsing fever transmitted indirectly by lice, and yaws where *Musca domestica* may at times convey the organism from diseased to healthy persons.

Notes to Table IV.—Diseases of Protozoal Origin

Both *direct* and *indirect* methods of transmission by insects occur with diseases of this class. With the intestinal parasites, indirect transmission takes place by the flies feeding on fæces containing the resistant stages (cysts), and later depositing them on human food and drinking water either by regurgitation of the stomach contents, or more often *per anum,* as Wenyon and O'Con-

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TABLE II.—(Continued.)

Organism	Host	Disease	Vector
Micrococcus melitensis ...	... Man and goats ...	Undulant fever; *syns.* Malta fever, Mediterranean fever, Remittent fever	[Flies]. Although the main channel of infection is the consumption of goat's milk
Diplococcus intracellularis ...	... Man	Cerebrospinal fever ...	Flies?
" *pemphigi contagiosi*	... "	Tropical impetigo ...	[Lice]

TABLE III.

THE MORE IMPORTANT INSECT-BORNE DISEASE OF SPIROCHÆTAL ORIGIN

N. B.—Names between square brackets = certain vectors; names without square brackets = probable vectors; names followed by ? = possible vectors.

The word "flies" includes in the main:—*Musca domestica, Fannia* spp., *Calliphora* spp., *Lucilia* spp., and *Sarcophaga* spp.

Organism	Host	Disease	Vector
Spirochæta carteri	Man ...	Indian relapsing fever	[Lice]
" *duttoni*	" ...	African " " (Tick fever) ...	[Ticks *(O. moubata, O. savigny)*]
" *gallinarum*	Fowls ...	Spirochætosis	[*Argas persicus*]
" *novyi*	Man ...	American relapsing fever	[Lice]
" *pertenuis*	" ...	Yaws (Framboesia)	Flies?
" *recurrentis*	" ...	European relapsing fever	[Lice]. Bed-bugs?
" *berbera*	" ...	North African relapsing fever ...	[Lice]

TABLE IV.

THE MORE IMPORTANT INSECT-BORNE DISEASES OF PROTOZOAL ORIGIN

N. B.—Names between square brackets = certain vectors; names without square brackets = probable vectors; names followed by ? = possible vectors.

The word "flies" includes in the main: *Musca domestica, Fannia* spp., *Calliphora* spp., *Lucilia* spp., and *Sarcophaga* spp.

Organism	Host	Disease	Vector
Entamœba histolytica ...	Man	Amœbic dysentery	[Flies]
Lamblia intestinalis ...	"	Flagellate "	[Flies]
Plasmodium malariæ ...	"	Quartan malaria	[Anopheline mosquitoes]
" *vivax* ...	"	Benign tertian malaria	[Anopheline mosquitoes]
" *falciparum* ...	"	Malignant or subtertian malaria ...	[Anopheline mosquitoes]

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nor have shown recently.[1] Needless to say, infection also occurs—and perhaps principally—by mechanical and ærial transmission of the cysts to food and water.

The majority of the protozoal blood parasites have insect vectors, on which they depend solely for transmission, and in certain cases these vectors are specific: Malaria, *Anophelines;* sleeping sickness, *Glossinæ;* European relapsing fever, *Pediculi.* Other insect-borne blood parasites are apparently able to be transmitted by more than one vector, i. e., Kala-azar, bed-bug (Patton); kala-azar, *Triatoma rubofasciatus* (Donovan); Souma (*Trypanosomiasis*), *Glossinæ;* Souma (*Stomoxys calcitrans*).

NOTES TO TABLE V.—DISEASES OF HELMINTHAL ORIGIN

With the exception of possible infection with certain helminths, resulting from the carriage by flies of helminth ova from fæces and subsequent deposition of the ova on food, the insect-borne helminths all undergo part of their life-history in the body of the insect vector. Thus the adult *Filaria bancrofti* live in human lymphatic glands. The ova find their way into the blood-stream, where they hatch to the Microfilariæ, and some are taken up from the blood when a mosquito bites a person harbouring the organisms. These, if they have entered the stomach of *Culex fatigans,* or other intermediate host, soon make their way to the thoracic muscles of the mosquito, where they undergo definite metamorphosis. When this is complete (usually in from sixteen to twenty days) the worms make their way into the mosquito's proboscis, and when next it pierces the skin of some victim the filariæ burst through the proboscis sheath and make their own passage through the skin, from which they soon travel to some lymphatic gland, where they become sexually mature, and the cycle is repeated. Similarly, *Dipylidium caninum* passes part of its life-history in the rat flea, and becomes sexually mature in the dog or man. The ova are ingested by the larval flea, and infection by the cysticercoid stage follows the accidental ingestion of the flea by the definite host.

[1] "The Carriage of Cysts of *Entamœba histolytica* and other protozoa, and eggs of Parasitic Worms by House-flies, with some Notes on the Resistance of Cysts to Disinfectants and other Agents." C. M. WENYON and F. W. O'CONNOR, *Journal of the Royal Army Medical Corps,* May, 1917, p. 522.

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TABLE IV.—(Continued.)

Organism	Host	Disease	Vector
Leishmania tropica	"	Oriental sore	Flies? Fleas? Phlebotomus? Hippobosca?
" *donovani*	"	Kala-azar	Bed-bugs? Fleas? Triatoma?
" *sp. incerta*	"	Espundia	Probably some tropical blood-sucking insect
" *infantum*	Children	Leishmaniasis	Fleas?
Trypanosoma gambiense	Man	Sleeping sickness	[*Glossina palpalis* (Tsetse flies)]
" *rhodesiense*	"	" "	[*Glossina morsitans*] " "
" *brucei*	Cattle and horses	Fly sickness (Nagana)	[*Glossina morsitans*] " "
" *lewisi*	Rats	Rat trypanosomiasis	Rat louse? and [Rat fleas *(Ceratophyllus fasciatus), Ctenocephalus canis*]
" *evansi*	Horses, mules, camels	Surra	[Horse flies (Tabanidæ), *Tabanus striatus*]
Schisotrypanum crusi	Man	Chaga's disease	[*Triatoma* (Conorhinus) *megistus*]
Babesia bigeminum	Cattle	Red-water fever	[Ticks *(Margaropus annulatus)*]
" *ovis*	Sheep	Piroplasmosis	[Ticks *(Rhipicephalus bursa)*]
" *canis*	Dogs	Malignant jaundice	[Ticks *(Rhipicephalus sanguineus* and *Hæmaphysalis leachi)*]
" *caballi*	Horses and mules	Piroplasmosis	[Ticks *(Dermacentor reticulatus)*]
Nuttallia equi	" "	"	[Ticks *(Rhipicephalus evertsi)*]
? Chlamydozoa ?	Man	Ophthalmia ægyptica and other ophthalmic conditions	[Flies]

*By some authorities *Lamblia intestinalis* is not regarded as pathogenic.

TABLE V.

THE MORE IMPORTANT INSECT-BORNE DISEASES OF HELMINTH ORIGIN

N. B.—Names between square brackets = certain vectors.

The word "flies" includes in the main: *Musca domestica, Fannia* sps., *Calliphora* sps., *Lucilia* sps., and *Sarcophaga* sps.

Organism	Host	Disease	Vector
Dipylidium caninum	Man and dogs	Tæniasis (tapeworm)	[Dog louse *(Trichodectes latus)*]; [Dog flea *(Ctenocephalus canis)*]; and the [Human flea *(Pulex irritans)*]
Ova of certain helminths	Man	Helminthiasis (parasitic worms)	[Flies]
Microfilaria bancrofti	"	Filariasis (Elephantiasis)	[Mosquitoes *(Culex* and *Anopheles* sps.)]
Loa loa	"	Calabar swellings	[Horse flies *(Chrysops dimidiata* and *Chrysops silacea)*]
Filaria immitis	Dogs	Dog filariasis	[Mosquitoes *(Culex* and *Anopheles* sp.)]
Hymenolepis diminuta	Rats, and occasionally man	Tæniasis	[Fleas]

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Table VI.

The more important Diseases directly attributable to Insects and Acarina

N. B.—Name between square brackets = certain vector.

Organism	Host	Disease	Vector
The larvæ of *Fannia canicularis* ...	Man	Intestinal myiasis	—
" " *Piophila casei* ...	"	" "	—
" " *Eristalis tenax* ...	"	" "	—
" " *Muscina stabulans* ...	"	" "	—
" " *Sarcophaga* sps. ...	"	Intestinal, dermal and muscular myiasis	—
" " *Lucilia* sps. ...	"	" " " " "	—
" " *Calliphora* sps. ...	"	" " " " "	—
" " *Chrysomyia macellaria* (the Screw worm)	" and animals ...	Nasal, auricular, and dermal myiasis ...	—
" " *Cordylobia anthropophaga* (the Tumbu fly)	Man	Dermal myiasis	—
" " *Dermatobia hominis* ...	"	" "	[Mosquito (*Janthinosoma lutzii*)]
" " " " ...	"	" "	
" " *Hypoderma bovis*, &c. ...	Cattle (occasionally man)	" " (Creeping disease in man)	—
" " *Œstrus ovis*	Sheep (rarely man)	Nasal myiasis	—
" " *Gastrophilus equi*, &c.	Horses	Gastric myiasis	—
" " *Trombidiidæ* (Harvest mites)	Man	Severe cutaneous irritation	—
Pediculoides ventricosus	"	Dermatitis	—
Tyroglyphus siro	"	" (the so-called "vanillism") ...	—
Other *Tyroglyphidæ*	"	" (Grocers' itch)	—
Tyroglyphus longior castellani ...	"	Copra itch	—
Sarcoptes scabiei	" and animals ...	"Itch" or "scabies"	—
Pediculus capitis	Man	Pediculosis of the head	—
" *humanus*	"	" " body	—
Phthirius pubis	"	" " axillary and pubic regions	—
Dermatophilus penetrans (Chiggers)	" and animals ...	Severe cutaneous irritation	—

The more important Insect-borne Diseases of Fungus Origin

N. B.—Name between square brackets = certain vector.

Organism	Host	Disease	Vector
Achorion schonleinii ...	Man ...	Favus ...	[Lice (Pediculi)]

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Notes to Table VI.—Diseases Directly Attributable to Insects and Acarina

In each case the disease results from the damage done by the insects and acarina in adopting existence upon the body of the host and living upon its tissues. The time spent upon the host may cover the whole life-history of the parasite and many subsequent generations, as with *Sarcoptes scabiei* and the *Pediculi,* or it may only embrace part of the parasite's development, as with the larvæ of flies causing myiasis. In either case the injury to the host may be so extensive as to cause death from the loss of vital tissue, or the injury itself, although insignificant, may indirectly cause death to the host by providing a suitable path for invasion by pathogenic micro-organisms.

Conclusion

It should be borne in mind that a large number of diseases included in the foregoing tables are not confined to being spread by insects, and insect transmission may in some cases only be occasional. This fact, however, cannot afford the preclusion of such diseases from consideration, and where transmission of the indirect type is possible, it is obvious that we are unable to form any just estimate of its relative importance. Probably, nevertheless, the dissemination of pathogenic micro-organisms by flies, for all that has lately been said in this connection, has not even yet been over-emphasized. It seems more than likely that *Bacillus tuberculosis* is spread in this manner from infective sputum to food (milk particularly) to a much greater extent than is commonly imagined, and there being no probability of rapid acute infection, as with *B. typhosus,* the part played by the fly is too apt to be overlooked.

Much of our knowledge with regard to insects and disease is still indefinite, as may be seen from the tables, but to anyone not particularly conversant with the subject, what we already know with certainty, even in connection with only the more important diseases that have been considered, may be sufficient to cause some little surprise.

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THE ACANTHOCEPHALA OF NORTH AMERICAN BIRDS*

H. J. VAN CLEAVE

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I. INTRODUCTION

Parasitologists have given little attention to the Acanthocephala parasitic in North American birds. The result has been a rather general belief that infestation with Acanthocephala in this class of vertebrates is rare. It is true that heavy infestations are more commonly found in other groups of vertebrates, but many of the

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water and shore birds carry very heavy infestations of Acanthocephala. Among the land birds, while infestation is not rare, it is usually of less frequent occurrence, and the number of parasites found in a single host individual is usually smaller. Most of the earlier records of the occurrence of Acanthocephala in North American birds ascribe the forms found to known European species. This is not surprising when one reviews the extent to which the study of Acanthocephala in birds has proceeded in Europe. Yet the mere fact that all earlier writers recognized but the one genus, Echinorhynchus, in part explains their lack of appreciation of specific characters within this group. On the whole, most of the earlier specific descriptions are little more than sufficient to permit of later workers recognizing the genus to which the species belong. This lack in specificity of earlier descriptions quite naturally lead the new world investigators to believe that the species they found on this continent were the same as the European species, since the descriptions published contained insufficient data to permit of a separation.

As indicated by the writer in an earlier paper (Van Cleave 1916 b: 228), the acanthocephalan fauna of North America is in the main a distinctive fauna with few species identical with those of the European fauna. The attempt on the part of earlier parasitologists to ascribe names of European species to forms found on this continent has, to a great extent, hindered the appreciation of this distinctness of the North American fauna, and at the same time has lead to considerable confusion regarding the geographical distribution of the genera and species of Acanthocephala. Unfortunately, many of the specimens of the older writers are not available for restudy to determine the correctness of the original determinations. However, in most cases where further study has been possible points of difference from the European species have been found too numerous to permit of including the American forms in the European species.

The writer has made an extensive study of the Acanthocephala of birds in which he has had access to the collections of the U. S. Bureau of Animal Industry, the U. S. National Museum, the Marine Hospital Service, private collections of Dr. H. B. Ward and of the

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writer. Papers covering the species of several genera of Avian Acanthocephala have already been published by the writer as a result of the study of these collections. The present article deals with the descriptions of several new species and a reconsideration of some forms previously described, but here for the first time ascribed to the proper genera as recognized by more recent developments of classification of the Acanthocephala. In all cases the study has been made from cleared, stained specimens mounted in damar.

Linton (1892: 92) recorded the occurrence of *Echinorhynchus striatus* Goeze from the intestine of the black scoter, *Oidemia americana,* collected at Yellowstone Lake, Wyoming. The writer has reexamined the original material of this collection and finds that these individuals constitute an undescribed species of the genus Corynosoma. Linton (page 92) remarked that the two females constituting the extent of the infestation of one of the hosts differed from the six males from the other host individual, in the "absence of spines at the posterior end" of the former. It is true that this sexual dimorphism is characteristic of the members of the genus Corynosoma as indicated by the founder of the genus, Lühe (1904 and 1911).

Of the materials from this collection deposited in the U. S. National Museum, one entire female, a portion of a second and five of the males, have been studied by the present writer. The entire female specimen is immature but, fortunately, the fragment of a specimen is of the anterior end of a fully mature individual so that the body cavity contains well developed embryos.

II. The Genus Corynosoma Lühe, 1904

The genus Corynosoma was established by Lühe to accommodate two species of Acanthocephala parasitic in fish-eating mammals and birds. This genus is characterized by the presence of spines on the anterior end of both sexes while in addition the males bear spines around the genital opening. The body is swollen anteriorly and with its coating of spines adheres to the intestinal mucosa of the host thereby furnishing an accessory means of attachment. Posteriorly the body gradually becomes smaller.

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Corynosoma constrictum nov. spec.

Plate I, Figures 1, 2 and 3

Synonym: *Echinorhynchus striatus* Goeze of Linton 1892.

SPECIFIC DEFINITION. With the characters of the genus. Body of the males 2.28 mm. to 4.3 mm. long, with a maximum diameter of from 0.5 mm. to 0.6 mm. Linton gives the measurement of a female 3.3 mm. long with a diameter of 0.8 mm. Proboscis slightly larger at base than at tip, armed with sixteen longitudinal rows of ten to twelve hooks each. Hooks near base of proboscis 0.035 to 0.041 mm. long, near middle of proboscis 0.041 to 0.047 mm. long, near tip of proboscis 0.030 to 0.041 mm. long. A constriction occurs around the body at about the anterior third (see Fig. 1). In both sexes the part of the body wall anterior to this constriction is armed with small cuticular spines about 0.030 mm. long. Each of these spines is embedded in a triangular elevation of the cuticula projecting from the general body surface. In the males there occurs in addition a group of cuticular spines surrounding the genital opening (Fig. 2). The genital spines are of the same size as those on the anterior region of the body, usually with the tip strongly recurved. Embryos in body of female 80 to 108μ long by 12 to 16μ wide (Fig. 3).

Type host *Oidemia americana,* in intestine. Type locality Yellowstone Lake, Wyoming. The cotypes of this species are deposited in the Smithsonian Institution; the males under catalog number 5449 and females under number 5439.

III. The Genus Plagiorhynchus Lühe, 1911

In the same paper mentioned above (1892: 91) Linton described another species of Acanthocephala from the intestine of a gull taken at Guaymas, Mexico. To this new species, founded on the study of one male and one female, he gave the name *Echinorhynchus rectus.* The present writer has examined the female of this collection, the only specimen in the bottle of material turned over to him for study from the collections of the U. S. National Museum. Facts brought out by the reexamination of the female of this species and the study of Linton's description of the male and female clearly indicate that this species belongs in the genus Plagiorhynchus, which

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up to the present time has not been recorded as occurring in North America. In the present paper the writer describes still another species belonging to the genus Plagiorhynchus.

CHARACTERS OF THE GENUS PLAGIORHYNCHUS. Acanthocephala belonging to this genus are parasitic as adults in the alimentary canal of birds. The proboscis is cylindrical with numerous hooks arranged in radial symmetry. The body proper, which is entirely devoid of spines, is usually short, elliptical, or with a tendency toward egg-shaped. The proboscis receptacle is a double walled muscular sac attached at the base of the proboscis. In dealing with other genera of Acanthocephala the writer has found that in Arhythmorhynchus (Van Cleave 1916:a) the embryos in recently discovered American species differ in appearance from those described for European species of obviously the same genus. Here again in the genus Plagiorhynchus Lühe (1911:27) described embryos and figured one having the middle membrane with a conspicuous spherical knob at each of its two poles. This he considered characteristic of the entire genus (1911:26). The embryos of *P. formosus* (Fig. 6) are elliptical with no polar knobs. Here is evidence, in addition to that previously brought out by the writer (1916:a) and mentioned above, regarding the advisability of omitting the shape of the embryonic shells from the generic diagnosis.

Lühe (1911:26) specified the presence of six closely compacted, thickset, cement glands as characteristic of the genus Plagiorhynchus. In earlier papers (1914 and 1916) the present writer has shown that shape and number of cement glands may both vary widely among different species of the same genus. Consequently I propose that a fixed number of cement glands and the shape of the cement glands be omitted from the list of characters diagnostic of this genus. Descriptions of two species, one new, and the other newly attributed to this genus follow.

Plagiorhynchus rectus (Linton, 1892)

Plate I, Figure 7

Synonym, *Echinorhynchus rectus* Linton, 1892

Described originally from one male and one female of which the female only has been reexamined by the present writer. Body

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of female 9 mm. long and 0.8 mm. in diameter. Proboscis cylindrical, 1.9 mm. long; 0.26 mm. in diameter; armed with twenty-four longitudinal rows of about twenty hooks each. In specimen examined this last number was calculated on the basis of the number of hooks on the exposed portion of the proboscis, since the tip of the proboscis is inverted. Hooks near the base of the proboscis 0.082 mm. long, with a diameter of 0.016 mm. at the point of emergence from proboscis wall; hooks near middle of proboscis 0.070 mm. long, recurved, with a diameter of 0.020 mm. at the point where the hooks curve backward; hooks near tip of proboscis 0.053 mm. long. Male 8.8 mm. long and 0.8 mm. in diameter. Testes oval, approximate, median. Female not fully mature so measurements of embryos cannot be given.

Type host, Larus (Chroicocephalus) sp. taken at Guaymas, Mexico. Type female deposited in U. S. National Museum, catalog number 5431.

Plagiorhynchus formosus nov. spec.

Plate I, Figures 4, 5 and 6

SPECIFIC DEFINITION. With the characters of the genus. Body about 10 mm. long, elliptical to slightly ovoid. Proboscis practically cylindrical, diameter about one-third of length; armed with sixteen longitudinal rows of thirteen to fourteen hooks each. Cement glands long, tubular. Hard shelled embryos inside body of female elliptical, 48 to 60μ long by 12 to 20μ in diameter.

This species is described from four mature individuals, two males and two females, in the Parasite Collection of the U. S. Bureau of Animal Industry; catalog number 4598. The writer designates one male and one female as types of the species. Descriptions of these follow.

TYPE MALE. Body elliptical with anterior and posterior extremities slightly flexed ventrally; entire length 8.5 mm.; maximum diameter 2 mm. Body proper devoid of spines. Proboscis subcylindrical, length 1.06 mm., diameter 0.33 mm., armature as mentioned in definition of species. The following table of measurements of hooks in a single row from tip to base of proboscis indicates relative size of hooks in various regions of proboscis.

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Row	1	2	3	4	5	6	7	8	9	10	11	12	13	
Length in μ.....71	77	83	83	83	83	83	83	83	77	77	77	65	Base	

Proboscis receptacle cylindrical, 1.73 mm. long, 0.42 mm. in diameter, base of the receptacle rounded, with invertors of proboscis penetrating posterior extremity (Fig. 4). Brain 0.180 mm. long, in center of proboscis receptacle, between the invertors. Retinacula conspicuous at the middle of the proboscis receptacle. Lemnisci 0.192 mm. long, 0.058 mm. in diameter. Anterior and posterior testes form an oblique line of contact; each 1.15 mm. long and 0.6 mm. in diameter. Cement glands long, tubular, extending from the dorsal margin of posterior testis to region of bursa.

TYPE FEMALE. Body more nearly cylindrical than type male, 9.5 mm. long, 2 mm. in diameter. Tip of proboscis slightly inverted, size and aramture practically identical with that described for male. Body filled with developing embryos surrounding and covering almost all of internal organs. Hard shelled embryos within type female rather variable in size, 40 to 60μ long by 12 to 20μ in diameter.

Type host, the flicker, *Colaptes auratus,* in intestine. The four specimens upon which the species is founded were collected at Bowie, Maryland, October 9, 1906, by Dr. B. H. Ransom.

MORPHOLOGY. The males of this species display a phenomenal transparency of body structures so that in stained whole mounts minute internal structures are observable with the greatest ease. The proboscis in the preserved specimens is so inserted that it points ventrally at an angle of approximately 60 degrees from the chief axis of the body. At the base of the proboscis the body proper is slightly expanded to form a thickened rim. The proboscis receptacle is a cylindrical sac composed of two muscular layers. The retinacula arise in the brain as two fairly small fibres. Upon penetration of the wall of the receptacle their size increases appreciably. This increased size is maintained through the remainder of their course to their insertion upon the body wall. The means of insertion upon the wall of the body is shown in Fig. 4. The lemnisci are slightly longer than the proboscis receptacle and of smaller diameter throughout.

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The two testes of the male are roughly oval, lying in the anterior region of the body. Lühe (1911:26) described six closely compacted cement glands as characteristic of the genus Plagiorhynchus. *P. formosus* appears to have six of these glands (Fig. 4), but their shape differs radically from the shape of the cement glands of *P. lanceolatus*.

The hooks upon the proboscis present a perfect pattern in their exact arrangement in alternating rows. Roots of the hooks are not distinct. The base of each hook for a length of about 0.021 mm. lies embedded in the wall of the proboscis perpendicular to its surface. The free portion of each hook is directed backward at an angle of about 110 degrees with the basal portion. The foregoing description of hooks applies to all hooks upon the proboscis except one or two hooks at the base of each row, which are simple, thornlike and one or two of those at the tip of the proboscis which differ from the remaining hooks in shape and general proportions (see Fig. 5). In all cases measurement of hooks here as in other papers by the writer the length of a hook is considered as the straight line connecting the tip of the hook with its extreme basal part.

IV. The Genus Polymorphus Lühe, 1911

Acanthocephala parasitic as adults in the intestine of birds. Posterior tip of the body rather broadly truncated. Anterior end of body swollen and separated from more attenuated posterior region by a constriction. Anterior region of the body spined. Proboscis usually cylindrical, frequently smaller at base.

Polymorphus obtusus nov. spec.

Plate II, Figs. 8, 9, 10, 11, and 12

Body with a slightly enlarged anterior region. This enlargement is set off by a constriction back of which the body again assumes a diameter equal to that of the anterior enlargement, then gradually decreases in size to the posterior tip which is more or less flexed ventrally. Posterior tip terminates bluntly (Fig. 11). Females 7.7 mm. long, males 4 to 5.5 mm. long. Proboscis in all specimens examined partially inverted, armed with about sixteen longitudinal rows of hooks. Anterior body region armed with small

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cuticular spines about 24μ long (Fig. 9). Embryos within body of female 60 to 80μ long and 20 to 24μ wide with one conspicuous outpocketing of the middle membrane at each pole (Fig. 10).

Type host *Anhinga anhinga* (water-turkey), in intestine. Type locality, Florida. Described from three specimens, one female and two males. Cotypes in U. S. National Museum, catalog number 2967.

Polymorphus sp?

Plate II, Fig. 13

In addition to *Polymorphus obtusus* another representative of this genus has been studied by the writer, but the single specimen, a male, is insufficient to permit of a description of the species. Record is given here with a drawing of the proboscis in order to facilitate comparison in case other specimens are found from the same host later. The host of this undescribed species in the hooded merganser, *Lophodytes cucullatus*. Specimen in the U. S. Bureau of Animal Industry Collection, catalog number 2808.

V. The Genus Centrorhynchus Lühe, 1911

Centrorhynchus spinosus Van Cleave, 1916

Plate III, Figs. 14 and 15

A single species of this genus has been described from North America. The writer (1916b) described *C. spinosus* from the intestine of the egret, *Herodias egretta*. For description see paper cited above.

VI. The Genus Mediorhynchus Van Cleave, 1916

Mediorhynchus papillosus Van Cleave

Plate III, Figs. 16, 17, 18 and 19

Mediorhynchus robustus Van Cleave, 1916

Plate IV, Figs. 20 and 21

This genus, which to the present time has been recognized only from North America, was created by the writer (Van Cleave 1916b) for three new species of Acanthocephala belonging evidently near to the genus Centrorhynchus, but having characteristics which

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prevented including them in that genus. At the time the description of this genus was published the writer failed to designate which of the species should be considered as type. *M. papillosus* is hereby designated as type of the genus Mediorhynchus.

Mediorhynchus papillosus has been recorded from *Myiochanes virens* (the wood pewee) and *Porzana carolina* (the sora). *Mediorhynchus robustus* has been found in *Icteria virens* (the yellow-breasted chat).

Mediorhynchus grandis was originally included within this same genus but a more thorough study of Kostylew's articles dealing with the genus Heteroplus has convinced the writer that the species *grandis* is, in reality, a member of the genus Heteroplus. Kostylew (1913: 532) considered Heteroplus as near to the genus Gigantorhynchus from which he claimed to have separated it. On the other hand de Marvel (1905: 217) considered *Echinorhynchus otidis*, one of the species upon which Kostylew founded his genus Heteroplus, as a synonym for *E. aluconis*. *E. aluconis*, through the careful work of Lühe (1911), became type of the genus Centrorhynchus. At the same time *Gigantorhynchus mirabilis* de Marvel, which Kostylew claimed also belonged to his genus Heteroplus, is clearly, on the basis of de Marvel's descriptions and figures, one of the Centrorhynchidæ. De Marvel's figures show clearly the constriction of the proboscis at the line of insertion of the receptacle with the coincident differentiation of hooks on the anterior and posterior regions of the proboscis. Kostylew (1913), in describing the proboscis receptacle of Heteroplus, referred to the anterior half as being twice the diameter of the posterior half. This condition is typical of the genus Mediorhynchus and serves as an additional argument for considering the genus Heteroplus as a member of the family Centrorhynchidæ and not of the Gigantorhynchidæ.

VII. The Genus Heteroplus Kostylew, 1914

With the characteristics of the family Centrorhynchidæ. Anterior and posterior regions of the proboscis bearing widely different numbers of longitudinal rows of hooks.

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Heteroplus grandis (Van Cleave, 1916)

Plate V, Figs. 27, 28 and 29

Synonym, *Mediorhynchus grandis* Van Cleave, 1916

This species, the description of which is given in Van Cleave 1916 b: 226, was described from *Quiscalus quiscula* (the purple grackle) and *Sturnella magna* (the meadow lark). More recently the writer has examined a specimen from *Corvus brachyrhynchus* (the crow) taken in Maryland which proves to belong to this same species.

Kostylew's contention that the genus Heteroplus belongs to the Gigantorhynchidæ caused the writer at the time of the description of this species to believe that its superficial and general resemblance to Heteroplus was not of great significance.

VIII. The Genus Filicollis Lühe, 1911

Filicollis botulus Van Cleave, 1916

Plate V, Figs. 30, 31, 32, 33 and 34

But a single species of this genus, *F. botulus,* has been recorded from North America. This species differs widely from *F. anatis* of Europe, especially in the shape of the proboscis of the female. Specific characteristics upon which this species was founded are given in an earlier paper (Van Cleave 1916). Since the species was described the writer has discovered younger males than he had seen at the time of the original description. Figure 30 shows the arrangement of the internal organs of one of these young males. The eider ducks, *Somateria dresseri* and *S. mollissima,* are the known hosts of the species.

IX. The Genus Arhythmorhynchus Lühe, 1911

Arhythmorhynchus uncinatus (Kaiser, 1893)

Arhythmorhynchus trichocephalus (R. Leuckart, Kaiser, 1893)

Arhythmorhynchus brevis Van Cleave, 1916

Plate IV, Figs. 22, 23 and 24

Arhythmorhynchus pumilirostris Van Cleave, 1916

Plate IV, Figs. 25 and 26

Kaiser in 1893 published a description of *Echinorhynchus uncinatus* Kaiser and *E. trichocephalus* R. Leuckart. The writer

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(1916a: 172) showed that these two species belong in reality to the genus Arhythmorhynchus and in the same paper described two additional species for this genus, *A. pumilirostris* and *A. brevis*, both from the intestine of the American bittern, *Botaurus lentigenosus*.

X. Species Inquirendæ and Species of Doubted Determination

Echinorhynchus pici collaris Leidy, 1850

Leidy described and recorded the occurrence of four Acanthocephalans from North American birds, but no one of these is the present writer able to locate with any degree of certainty within the modern system of classification of the group. The description of *Echinorhynchus pici collaris* from *"Picus colaris"* is lacking in details sufficient to permit of speculation as to even the genus to which it belongs. Consequently *E. pici collaris* must be regarded as a *species inquirenda*. The other three species, given the names of known European species, probably belong to the respective genera now recognized as including the European forms.

(?) *Echinorhynchus caudatus* Zeder of Leidy 1887

This species, which Leidy recorded from *Elanoides forficatus* and from *Scotiaptex nebulosa*, has been considered by the present writer (1916b: 222) as belonging to the family Centrorhynchidæ, though the original description is insufficient to permit of a closer determination. It is extremely improbable that Leidy's determination of this species is correct.

(?) *Echinorhynchus striatus* Goeze of Leidy 1856

From the intestine of *Mycteria americana* (*"Tantalus loculator"*), the wood ibis, Leidy recorded the occurrence of *Echinorhynchus striatus* Goeze. Lühe (1911) was unable to place Goeze's *E. striatus* in his revised system of classification. It seems improbable that Leidy was dealing with the species which Goeze described from central Europe. Many points of detail have caused the present writer to consider the form which Leidy recorded as probably one of the species of Arhythmorhynchus. Its relations to other species of this genus cannot be determined.

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(?) *Echinorhynchus hystrix* Bremser of Leidy 1887

Leidy's report of *Echinorhynchus hystrix* from *Anhinga anhinga* (the water turkey) gives some ground for attributing the genus Corynosoma as parasitic upon that species of bird, though here again the specific identity with the European form is to be seriously doubted.

XI. Distribution of the Acanthocephala of Birds

It seems worth while to call attention to the fact that in the collections from birds examined by the writer the occurrence of two different species of Acanthocephala within the same host individual has never been observed. Furthermore there is no positive case on record wherein two different genera of Acanthocephala have been found in the same species of North American bird. The case of *Anhinga anhinga* seems to be an exception to this last statement. From this host the writer has described a species of Polymorphus while Leidy has described what seems to be a species of Corynosoma. However in general body form these two genera resemble one another closely enough to confuse the casual observer. On this basis the apparent exception to the general condition may not be a real one.

De Marval, in his significant monographic contribution to the study of Avian Acanthocephala, has unfortunately failed to furnish us with any considerable body of data upon the geographical distribution of the species with which he worked. Evidently many of his records are compilations from earlier writers. In his treatment of each species of Acanthocephala he has given a host list, but offers no information as to the locality from which the hosts were taken. A study of his data shows that he has recorded the occurrence of Acanthocephala in more than forty families of birds representing eleven of the seventeen orders of birds recognized in North America and in addition some families not represented in the North American fauna.

In the collections available to the writer these parasites have been found in but seven orders, and a total of but ten families of birds. Beyond this Leidy's reports indicate the presence of Acanthocephala in at least three additional families. It is a noteworthy

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fact that the geographical distribution of the species known to North America is restricted almost exclusively to the eastern part of the continent. In the opinion of the writer this apparent localization of infestation is probably due to the fact that records from the West are wanting rather than that the actual distribution is so narrowly limited. Much of the materials studied by the writer has been the result of the careful work of Mr. Albert Hassall.

XII. Distribution of Genera of Acanthocephala in Families of European and North American Birds:

Family of birds acting as host	Genera of Acanthocephala reported from Central Europe (Lühe 1911)	North America
Colymbidæ	Filicollis	
Laridæ	Filicollis	Plagiorhynchus
Anhingidæ		Polymorphus
		?Corynosoma
Anatidæ	Polymorphus	Polymorphus
	Filicollis	Filicollis
	Corynosoma	Corynosoma
	?Centrorhynchus?	
Ciconiidæ		?E. striatus?
Ardeidæ	Filicollis	Centrorhynchus
	?Arhythmorhynchus?	Arhythmorhynchus
Rallidæ	Polymorphus	Mediorhynchus
	Filicollis	
Scolopacidæ	Plagiorhynchus	
	Arhythmorhynchus	
	Filicollis	
Charadriidæ	Plagiorhynchus	
	Polymorphus	
Buteonidæ		?E. caudatus?
Falconidæ	Centrorhynchus	
Strigidæ		?E. caudatus?
Picidæ		Plagiorhynchus
		?E. pici collaris?
Tyrannidæ		Mediorhynchus
Corvidæ		Plagiorhynchus
		Heteroplus
Icteridæ		Heteroplus
Mniotiltidæ		Mediorhynchus

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In the above table genera and species marked with a question mark (?) are of questioned original determination or are *species inquirendæ*. It is quite a striking fact that in only the family Anatidæ and probably the Ardeidæ are any of the same genera of Acanthocephala found in both North America and Europe.

Lühe (1911), in his register of the Acanthocephala and parasitic flatworms from central European hosts recorded the occurrence of Acanthocephala from hosts representing but eight families of birds. The accompanying table based upon Lühe's data and records of the writer shows in striking manner the differences between the types of hosts characteristic of the various genera in Europe and North America. To the writer this table furnishes still farther evidence of the independence of the acanthocephalan fauna of the two continents. In the majority of cases even the genera of these parasites have found hosts in entirely different Families and even Orders of birds.

XIII. GENERA OF ACANTHOCEPHALA WITH THE ORDERS OF BIRDS FROM WHICH TAKEN

GENUS OF ACANTHOCEPHALA	ORDER OF BIRDS SERVING AS HOST	
	CENTRAL EUROPE (Lühe)	NORTH AMERICA
Arhythmorhynchus	Limicolæ	Herodiones
Centrorhynchus	Raptores	Herodiones
	Anseres	
Corynosoma	Anseres	Anseres
		?Steganapodes
Filicollis	Anseres	Anseres
	Pygopodes	
	Longipennes	
	Herodiones	
	Paludicolæ	
	Limicolæ	
Heteroplus		Passeres
Mediorhynchus		Passeres
		Paludicolæ
Plagiorhynchus	Limicolæ	Pici
		Longipennes
		Passeres
Polymorphus	Limicolæ	Steganapodes
	Anseres	Anseres
	Paludicolæ	

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A comparison of infestation within the *Orders* of birds furnishes a contrast between the two continents even more striking than that found in the comparison of the Families of birds. The accompanying table shows among other things, that Corynosoma, Filicollis and Polymorphus are the only genera having hosts within the same orders of birds on the two continents.

XIV. Key to the Genera and Species of Acanthocephala of North American Birds

1 (12) Acanthocephala with body proper devoid of spines..... 2

2 (5) Proboscis receptacle inserted at base of proboscis..... ..... Genus PLAGIORHYNCHUS.. 3

3 (4) Twenty-four longitudinal rows of hooks..... ..... *Plagiorhynchus rectus.* Linton

4 (3) Sixteen longitudinal rows of hooks..... ..... *Plagiorhynchus formosus* nov. spec.

5 (2) Proboscis receptacle inserted at or near middle of proboscis. Proboscis hooks anterior to and posterior to insertion conspicuously unlike 6

6 (7) Invertors of proboscis extending through posterior tip of proboscis receptacle and continuing as retractors for receptacle..... .....Genus CENTRORHYNCHUS

Centrorhynchus spinosus Van C., only known species in North America.

7 (6) Invertors of proboscis passing through wall of proboscis receptacle near its middle or at least considerable distance anterior to the posterior end 8

8 (9) Anterior and posterior regions of proboscis bearing different numbers of longitudinal rows of hooks.....Genus HETEROPLUS

Twelve longitudinal rows of hooks on the interior region of proboscis, about thirty on posterior. *Heteroplus grandis* (Van. C.) only known species in North America.

9 (8) Anterior and posterior regions of proboscis bearing the same number of longitudinal rows of hooks. Genus MEDIORHYNCHUS..10

10 (11) Proboscis not covered with conspicuous papillæ in which hooks are embedded. Body usually robust, forms with twenty-four longitudinal rows of hooks on proboscis. Embryos 38μ by 16μ..... .....*Mediorhynchus robustus* Van C.

11 (10) Proboscis hooks not conspicuous; embedded in conspicuous papillæ. Eighteen longitudinal rows of hooks. Embryos 38μ to 47μ by 18μ to 24μ.....*Mediorhynchus papillosus* Van C.

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12 (1) Body proper bearing spines, at least in restricted regions.....13

13 (14) Body spines on anterior region of the body and also surrounding genital opening.....Genus CORYNOSOMA, males

But one species, *Corynosoma constrictum* nov. spec., known to North America.

14 (13) With no cuticular spines around the genital opening.....15

15 (26) Anterior end of the body decidedly larger than posterior end.....16

16 (23) Anterior and posterior regions of the body of distinctly different histological structure.....Genus ARHYTHMORHYNCHUS..17

17 (18) Hooks on mid-ventral surface of proboscis conspicuously larger than on any other part of proboscis.....
.....*Arhythmorhynchus trichocephalus* (R. Leuckart)

18 (17) Hooks on ventral surface of proboscis not conspicuously larger than on any other surfaces.....19

19 (20) Longest hooks more than 100μ long.....
.....*Arhythmorhynchus uncinatus* (Kaiser)

20 (19) Longest hooks not more than 50μ long.....21

21 (22) Proboscis with sixteen longitudinal rows of hooks. Embryos 65 to 80μ long, 18μ wide....*Arhythmorhynchus pumilirostris* Van C.

22 (21) Proboscis with eighteen longitudinal rows of hooks. Embryos 76 to 100μ long, 24 to 30μ wide....*Arhythmorhynchus brevis* Van C.

23 (16) Anterior end of body larger than posterior end, but body wall of same histological structure throughout.....24

24 (25) Anterior region of body set off from posterior region by conspicuous constriction. Proboscis cylindrical, or frequently tapering slightly toward base.....Genus POLYMORPHUS

Polymorphus obtusus nov. spec. only species described from North America.

25 (24) Anterior region of body distinctly swollen, but not separated from posterior by a constriction. Proboscis spindle shaped.....
..... Genus CORYNOSOMA, females

Corynosoma constrictum nov. spec., only known species from North America.

26 (15) Body sac-like or sausage shaped, not conspicuously swollen at anterior end. Proboscis spherical or ovate, followed by a neck fully as long as the proboscis and sharply set off from the body proper.
.....Genus FILICOLLIS

Filicollis botulus Van C. is the only species known to North America.

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XV. Summary

1. This article contains the results of a study of the Acanthocephala parasitic in birds from the U. S. Government collections and the private collections of Professor H. B. Ward and of the writer.

2. *Echinorhynchus striatus* taken by Linton from *Oidemia americana* belongs to the genus Corynosoma and here is described as *Corynosoma constrictum* nov. spec.

3. *Echinorhynchus rectus* Linton, 1892 is shown to belong to the genus Plagiorhynchus. *P. formosus* nov. spec. from *Colaptes auratus* is described. These two species constitute the first record of species of Plagiorhynchus in North America.

4. *Polymorphus obtusus* nov. spec. is described from *Anhinga anhinga.*

5. *Polymorphus species?* is recorded from *Lophodytes cucullatus.*

6. *Mediorhynchus papillosus* Van C., 1916 is designated as type of the genus Mediorhynchus.

7. The genus Heteroplus is one of the Centrorhynchidæ (not of the Gigantorhynchidæ as maintained by Kostylew, its creator).

8. *Mediorhynchus grandis* Van C., 1916 is shown to belong to the genus Heteroplus.

9. *Corvus brachyrhynchus* is cited as a new host for *Heteroplus grandis* (Van C.).

10. Among North American birds the occurrence of two different species of Acanthocephala within the same host individual has never been recorded.

11. There is no positive case on record of the occurrence of two different genera of Acanthocephala within the same species of North American bird.

12. Tables are given to show the comparison of acanthocephalan infestation in the families and orders of birds of central Europe and of North America.

13. A key to all described species of Acanthocephala from North American birds is given.

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XVII. Explanation of Plates

All figures excepting Figure 7 are drawn by the aid of a camera lucida from stained specimens mounted in xylol damar. The magnification is indicated by the projected scale accompanying each drawing.

Abbreviations Used

b. bursa copulatorix
br. brain.
c.g. cement glands
e. egg masses
ins. insertion of proboscis receptacle
i.p. invertors of proboscis
l. lemniscus
p.r. proboscis receptacle
r. retinacula
r.p. retractors of proboscis receptacle
t.a. anterior testis
t.p. posterior testis

Plate I.

Figs. 1 to 3, *Corynosoma constrictum* nov. spec.

Fig. 1. Entire male showing general body shape and arrangement of internal organs.

Fig. 2. Posterior tip of male showing cuticular spines.

Fig. 3. Embryo from body of mature female.

Figs. 4 to 6, *Plagiorhynchus formosus* nov. spec.

Fig. 4. Entire male showing general body form and arrangement of internal organs.

Fig. 5. Profile of the proboscis of same individual as shown in Fig. 4.

Fig. 6. Embryo from body of mature female.

Fig. 7. *Plagiorhynchus rectus* (Linton). Drawing copied from Linton 1892.

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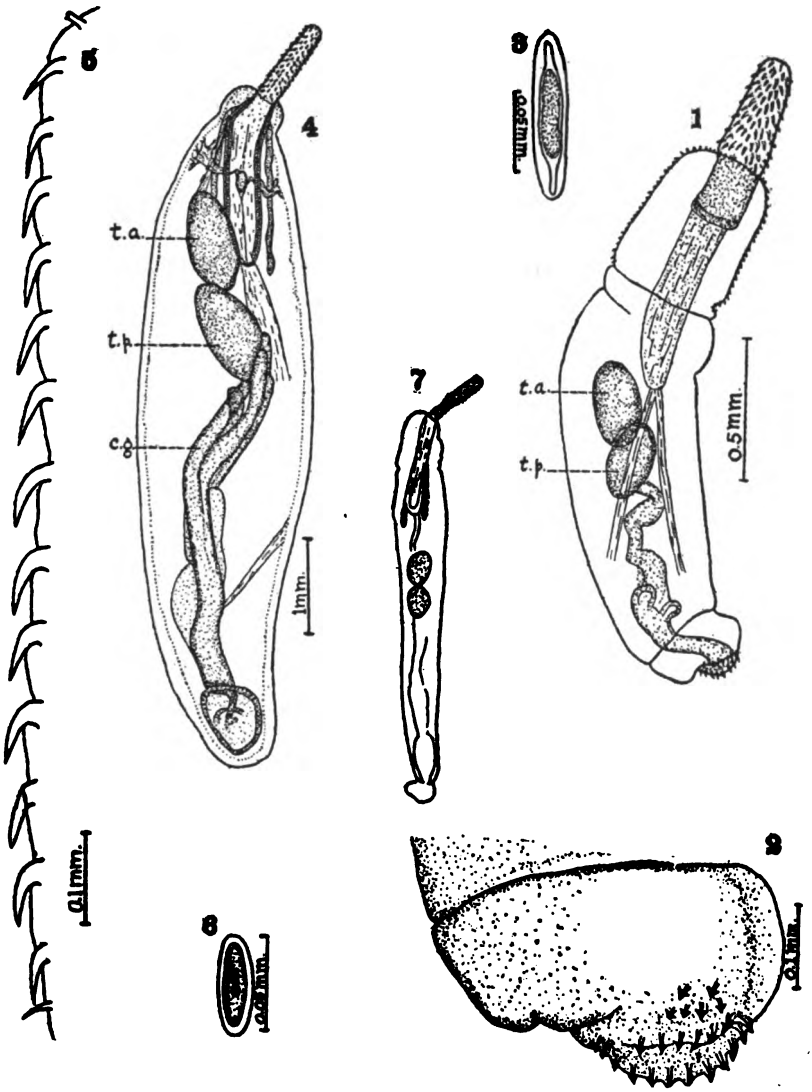

PLATE I.

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PLATE II.

Figs. 8 to 12. *Polymorphus obtusus* nov. spec.

Fig. 8. Entire male, poorly preserved specimen, internal organs very indistinct.

Fig. 9. Cuticular spines in profile, from anterior region of body.

Fig. 10. Embryo from body cavity of female.

Fig. 11. Posterior extremity of type female showing characteristic bluntness upon basis of which the specific name is given.

Fig. 12. Profile of proboscis hooks.

Fig. 13. *Polymorphus species?* from *Lophodytes cucullatus.*

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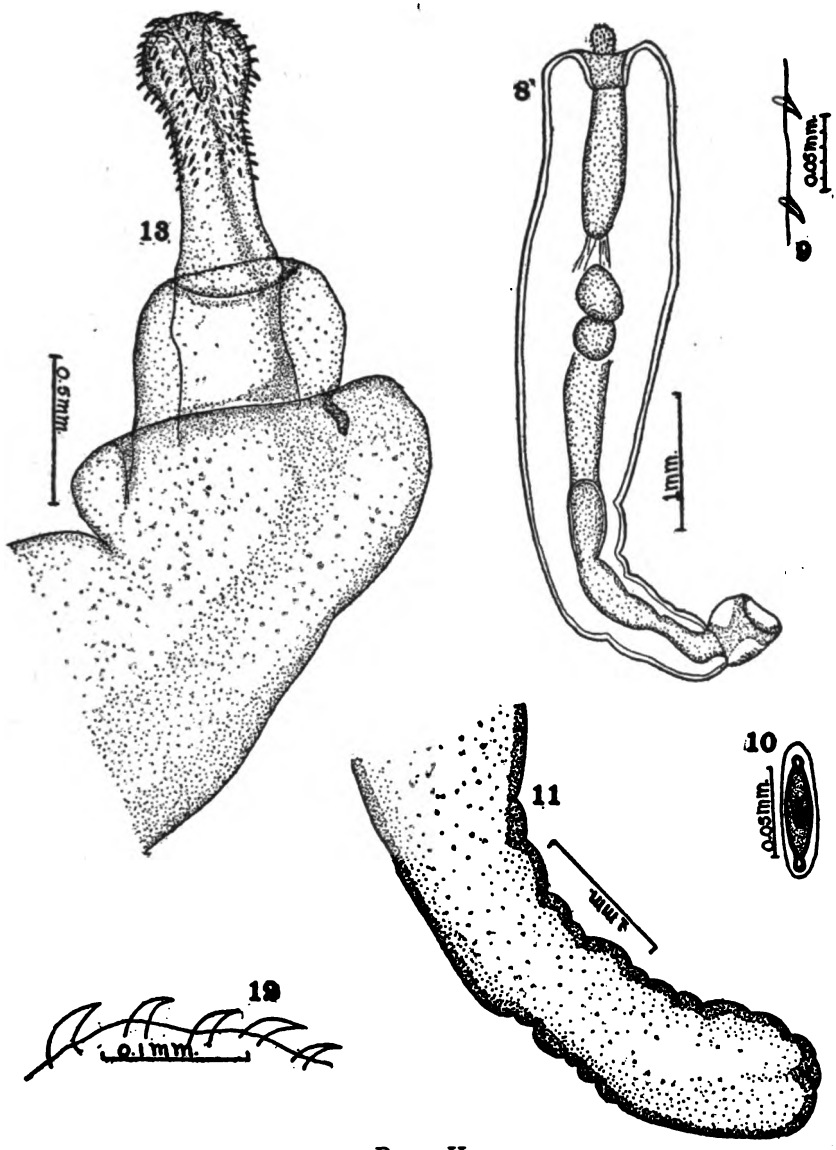

PLATE II.

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Plate III.

Figs. 14 and 15. *Centrorhynchus spinosus* Van Cleave.

Fig. 14. General body form of type female.

Fig. 15. Profile of dorsal surface of proboscis shown in Fig. 14.

Figs. 16 to 19. *Mediorhynchus papillosus* Van Cleave.

Fig. 16. Entire male showing general body form and arrangement of organs.

Fig. 17. Proboscis and anterior body region of type male.

Fig. 18. Profile dorsal surface of proboscis, same individual as shown in Fig. 17.

Fig. 19. Embryos from body cavity of fully mature female.

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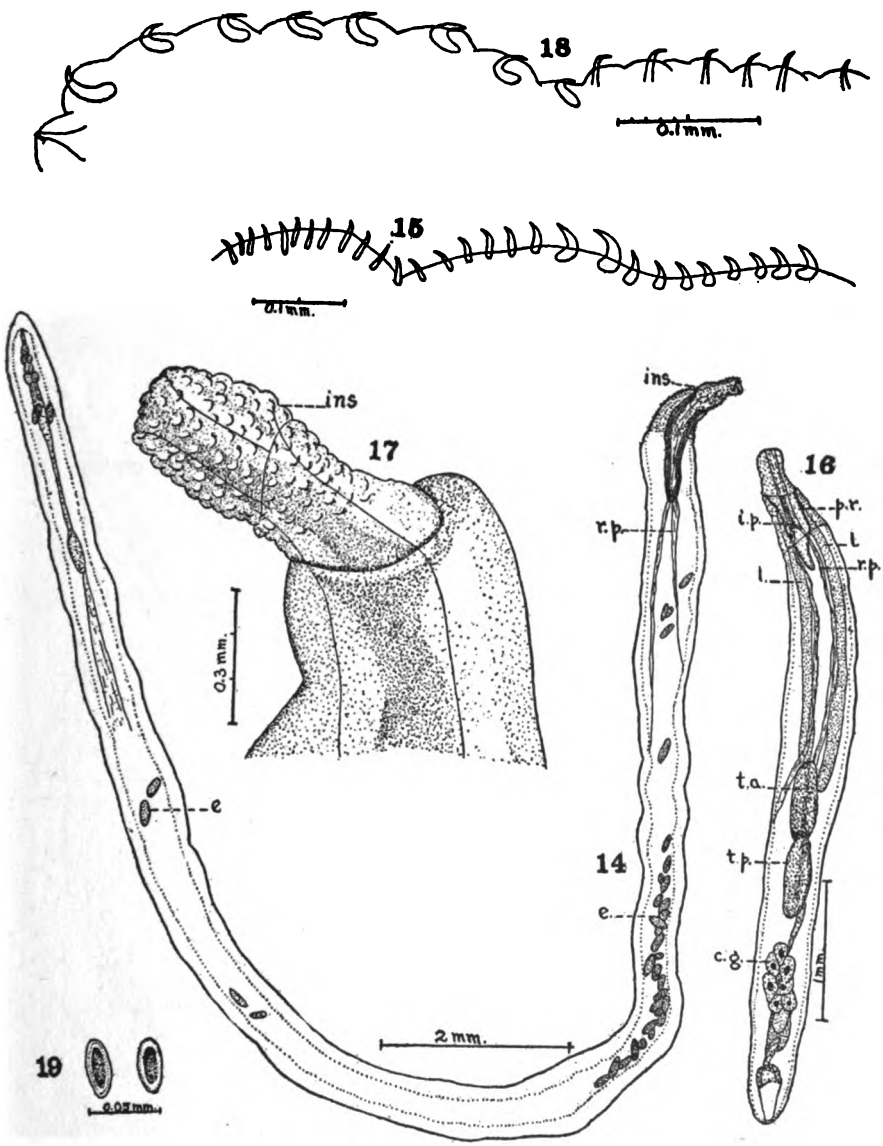

PLATE III.

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PLATE IV.

Figs. 20 and 21. *Mediorhynchus robustus* Van Cleave.

Fig. 20. Male in optical section showing body form and arrangement of organs.

Fig. 21. Embryos from body cavity of mature female.

Figs. 22 to 24. *Arhythmorhynchus brevis* Van Cleave.

Fig. 22. Body of entire male.

Fig. 23. Proboscis and anterior region of body of a male.

Fig. 24. Embryos from body cavity of mature female.

Figs. 25 and 26. *Arhythmorhynchus pumilirostris* Van Cleave.

Fig. 25. Entire male.

Fig. 26. Embryo from body of mature female.

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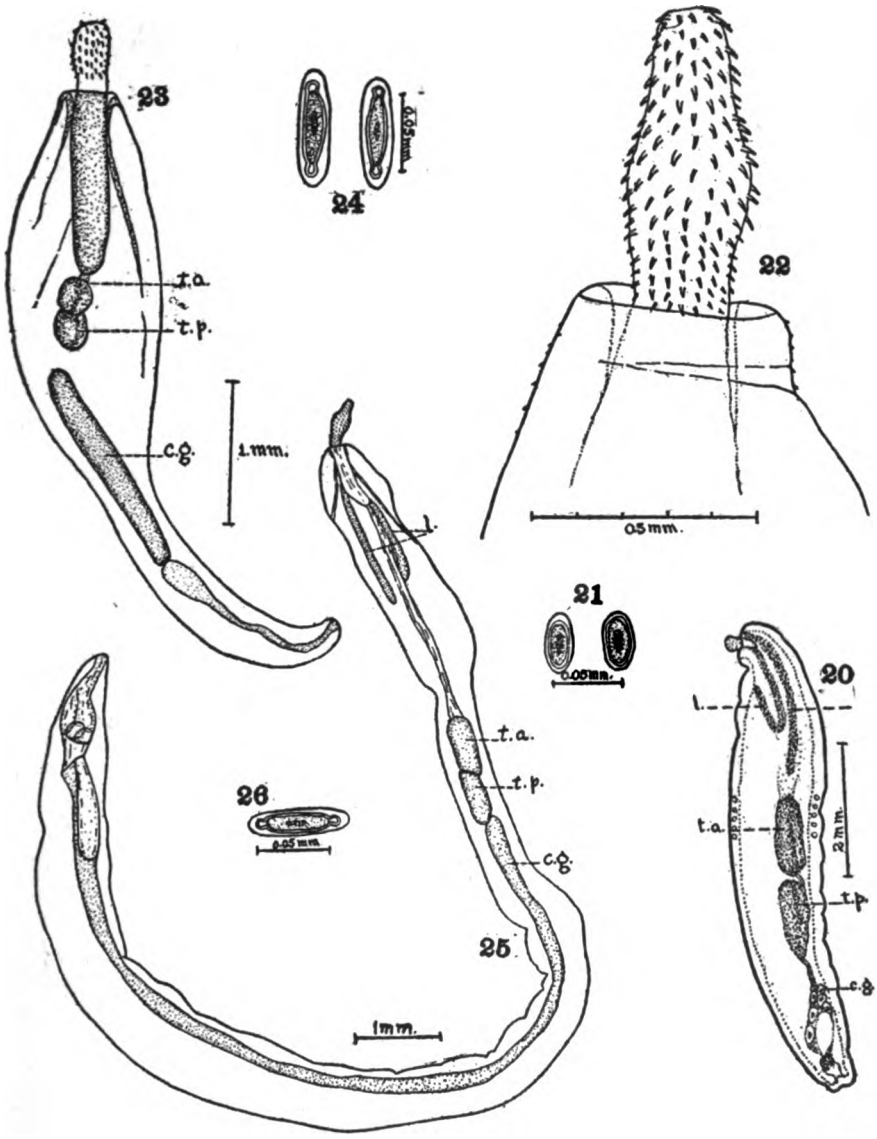

PLATE IV.

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PLATE V.

Figs. 27 to 29. *Heteroplus grandis* (Van Cleave).

Fig. 27. Proboscis partly invaginated, but showing the hook arrangement typical for the genus.

Fig. 28. Embryos from body of mature female.

Fig. 29. Profile of dorsal surface of proboscis.

Figs. 30 to 34. *Filicollis botulus* Van Cleave.

Fig. 30. Entire male showing general body form and arrangement of organs.

Fig. 31. Cuticular spines from anterior region of body.

Fig. 32. Mature female showing general body form.

Fig. 33. Embryo from body cavity of mature female.

Fig. 34. Profile, ventral surface, of proboscis of female.

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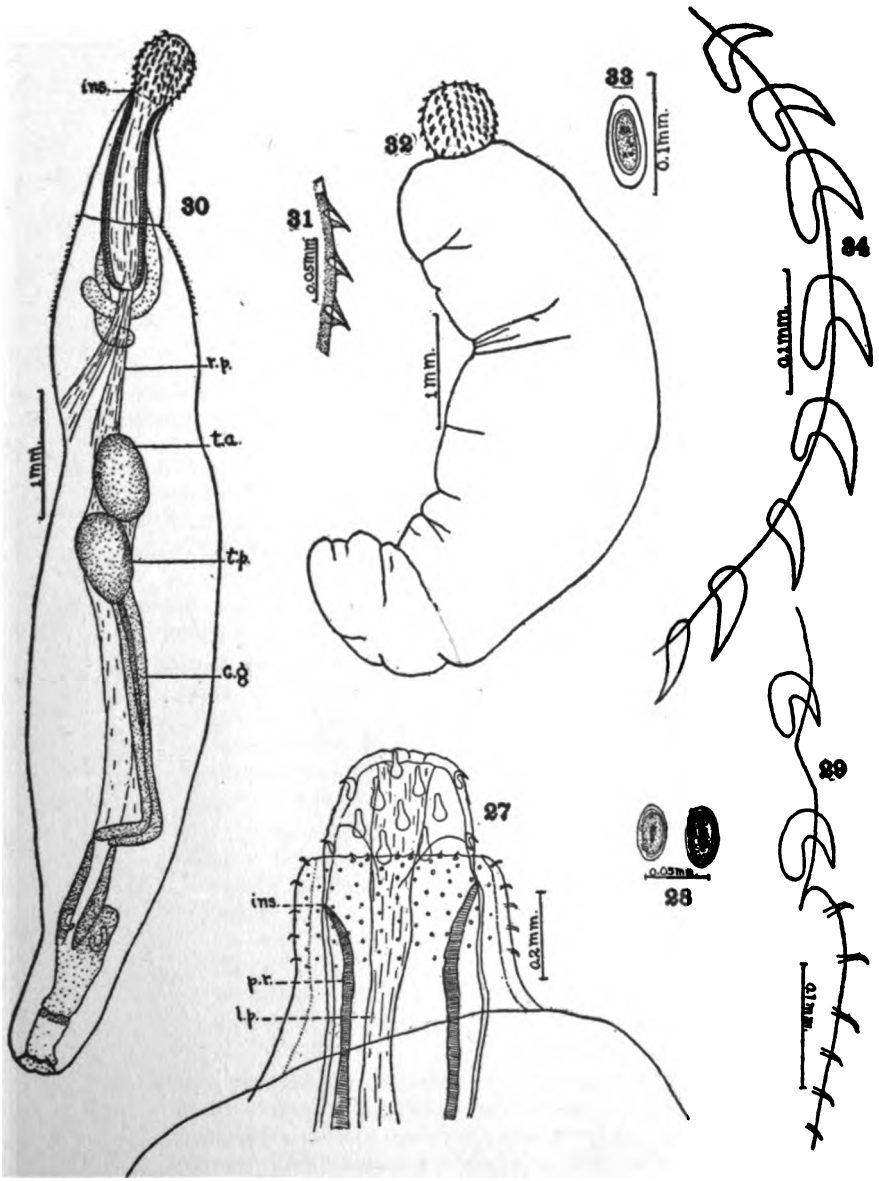

PLATE V.

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BRANCHIOBDELLID WORMS (ANNELIDA) FROM MICHIGAN CRAWFISHES

By MAX M. ELLIS

Through the courtesy of the Michigan Fish Commission the writer visited several islands in Potagannissing Bay and Lake Huron on Patrol Boat No. 4, Captain Robert E. Ellsworth commanding, during August, 1917. The branchiobdellid worms listed below were taken from crawfishes collected on this trip and from those of two additional collections made by Captain Ellsworth. Dr. Walter Faxon of Harvard University kindly identified the crawfish hosts.

The four species of branchiobdellids represented may be distinguished by the following key.

a. Anterior nephridia opening to the outside through two separate pores in segment III; segments VIII and IX each bearing a pair of glandular disks on the ventral surface; dental formula usually 5-4 or 5-5, the middle tooth of each jaw being the longest tooth in the jaw.

Xironodrilus formosus Ellis *in ed.*

aa. Anterior nephridia opening to the outside through one common pore in the dorsal surface of segment III.

b. Body segments without conspicuously elevated portions; accessary sperm tube present.

c. Upper and lower lips entire excepting a small, median emargination; dental formula 5-4, teeth approaching a subequal condition, although the middle tooth of the upper jaw is distinctly longer than the other four teeth of that jaw.

Cambarincola vitrea Ellis *in ed.*

cc. Upper lip composed of four, subequal lobes, which can be extended as digitiform processes; lower lip composed of two, subequal lobes which can be extended; a small, lateral lobe on each side at the junction of the upper and lower lips; dental formula 5-4; middle tooth of upper jaw long and prominent; lateral teeth small, not more than half the length of the middle tooth.

Cambarincola philadelphica (Leidy)

bb. Dorsal portions of segments elevated; segments VII and VIII with funnel-shaped enlargements of the dorsal portions of the segment, more or less completely encircling the segment; funnel of segment VIII excavated dorsally so that its dorsal margin bears two, small "horns"; dental formula 5-4.

Pterodrilus durbini Ellis *in ed.*

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(1) Xironodrilus formosus Ellis

From *Cambarus virilis* Hagen

1. James Island, Potagannissing Bay, August 4.
2. Three miles up Potagannissing River, Drummond Island, Potagannissing Bay, August 4.
3. Pilot Harbor, Sitgreaves Bay, north side of Drummond Island, Potagannissing Bay, August 6.
4. Little Cass Island, head of Detour Passage, August 6.
5. Churchville Point, head of Lake George, 46° 31′ N., August 7.
6. Harbor Island, Potagannissing Bay, August 8.
7. Winona Slips, Bay City, Saginaw Bay, September (Capt. Ellsworth).

(2) Cambarincola vitrea Ellis

From *Cambarus virilis* Hagen

1. James Island, Potagannissing Bay, August 4.
2. Three miles up Potagannissing River, Drummond Island, Potagannissing Bay, August 4.

From *Cambarus propinquus* Girard

1. Sault Sainte Marie, St. Marys River, August 7.
2. Echo Lake, Grand Island, Lake Superior, August 17 (Capt. Ellsworth).

(3) Cambarincola philadelphia (Leidy)

From *Cambarus virilis* Hagen

1. James Island, Potagannissing Bay, August 4.
2. Pilot Harbor, Sitgreaves Bay, north side of Drummond Island, Potagannissing Bay, August 6.
3. Little Cass Island, head of Detour Passage, August 6.
4. Harbor Island, Potagannissing Bay, August 8.

(4) Pterodrilus durbini Ellis

From *Cambarus virilis* Hagen

1. James Island, Potagannissing Bay, August 4.
2. Three miles up Potagannissing River, Drummond Island, Potagannissing Bay, August 4.
3. Pilot Harbor, Sitgreaves Bay, north side of Drummond Island, Potagannissing Bay, August 6.
4. Little Cass Island, head of Detour Passage, August 6.
5. Churchville Point, head of Lake George, 46° 31′ N., August 7.
6. Harbor Island, Potagannissing Bay, August 8.
7. Winona Slips, Bay City, Saginaw Bay, September (Capt. Ellsworth).

It may be seen from this list that *Xironodrilus formosus* Ellis and *Pterodrilus durbini* Ellis were found in every collection from

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Cambarus virilis Hagen. In each case *Xironodrilus formosus* was the more abundant species, represented by hundreds of individuals. Comparatively few *Pterodrilus durbini* were taken. The relative abundance of these two species may be considered as accurate for these collections as the living crawfish were dropped into the killing fluid as soon as caught, and all of the branchiobdellid worms carried by each crawfish preserved. The two species of *Cambarincola,* if found, were represented by a fair number of individuals. *Cambarincola vitrea* Ellis was the only species taken from *Cambarus propinquus* Girard in these collections. Both species of *Cambarincola* here represented however have been taken from specimens of *Cambarus propinquus* at Douglas Lake, Michigan.

University of Colorado.

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DEPARTMENT OF NOTES, REVIEWS, ETC.

It is the purpose, in this department, to present from time to time brief original notes, both of methods of work and of results, by members of the Society. All members are invited to submit such items. In addition to these there will be given a few brief abstracts of recent work of more general interest to students and teachers. There will be no attempt to make these abstracts exhaustive. They will illustrate progress without attempting to define it, and will thus give to the teacher current illustrations, and to the isolated student suggestions of suitable fields of investigation.—[Editor.]

A CHART ON GENERAL PLANT HISTOLOGY AND PHYSIOLOGY

The valuable teaching aid afforded by charts and diagrams of various sorts is well understood by most teachers of biology who are more or less well acquainted with the numerous current sets of charts offered to the profession. There are a few teachers who possess the enviable talent of rapidly constructing excellent blackboard sketches during a given lecture or laboratory period to illustrate the particular features or phenomena under study at that particular time. Some instructors have made use of the more permanent crayon sketches on sheets of drawing paper hung over an easel. The uses of the various lantern-slide, opaque, vertical and micro-projection possibilities are also utilized to a very desirable degree under certain circumstances.

The principal pedagogical difficulty which all teachers have probably experienced in the practical application of these or other useful adjuncts of the same general kind to teaching is, that at best the student gets a disjointed presentation of the subject in question for the reason that the subject matter must be presented more or less disjointedly and interruptedly because of the many other things which we compel him to study. The student suffers from this regular lack of continuity. He loses much because of his failure to see the real position of a given structure or place of a given activity in the organism as a whole.

I may illustrate my meaning here by a reference to the common practice in teaching some of the phases of botany, say phytohistology. Probably students may be found in every class in plant histology as that subject is currently taught, who, knowing right well the detailed characteristics of all of the common tissues of the vegetable kingdom and how to handle the histological technique involved

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in the preparation of such tissues, may fail utterly to acquire an adequate knowledge of the place and function of those tissues in the plant as a whole. In fact such students may not even know where in the plant to find a given tissue if they are thrown upon their own resources, resources derived from their histology courses. Surely it is most difficult for them to really understand the function of the various tissues of the plant if they do not know the position of the tissues within the plant.

The same general state of affairs sometimes exists in plant physiology. The student may understand the various fundamental processes of the plant very well, but he often fails to read through the whole series of interrelated activities and to visualize, as it were, the individual plant as a completely equipped and working entity.

The most fundamental process in all nature is photosynthesis. Consequently it is quite desirable that the student of general biology, of general botany, surely of general physiology and general science should understand at least the fundamental features and the significance of that great phenomenon. He should know photosynthesis as it occurs in the leaf, of course, but he should also know about many of the other processes and structures which make the photosynthetic manufacture of carbohydrates in the leaf possible. He must know quite well many of the interrelations of the various activities of the plant if he wishes to really understand photosynthesis. He must see the plant as a completely constructed mechanism with its various parts working together in harmony. It is the business of the teacher to see to it that he does get this notion definitely outlined in his mind.

Now to be sure that our students really *get* all of this and this point of view of the plant, requires more than a little thought on the part of the teacher as to the methods of instruction that are to be followed in the class-room and laboratory. Sometimes I have thought that some of the common difficulties in this connection might be lessened if the class were to take a single common plant, say the sunflower, castor bean or scarlet runner, and work out its entire structural and physiological life-history from the embryo in the seed through the development and maturation of the new individual

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including the new fruit and seed. If the exercises on histology and physiology were properly correlated at every point possible I believe that such a method would result in giving to the student an admirable introduction to many of the fundamentals of general botany. At the conclusion of such a course the student would have secured an admirable and useful insight into the most of the important plant phenomena. And I believe that he would actually retain a definite mental picture of what the plant *is* as a *mechanism* and what it *does* as a *living, working* organism. From such a course he would surely obtain a connected view of these matters and he would not think of the plant as composed of a group of parenchyma cells here, a strand of fibers there, an irregular patch of epidermis somewhere else. He would not stop to wonder how and from whence the root gets the necessary food for its growth or how it is possible for growth to begin in a tree in early spring before the leaves have unfolded their chlorophyll-containing tissues.

Something of the same results may be secured by the skillful teacher from a carefully planned summary of the whole matter in which the various interrelations and correlations are plainly worked out. I have found in my own department that generalized drawings aid greatly in connection with such summaries. An illustration which brings before the student by means of a single chart or page many of the essentials has been found most useful by myself and the other instructors in my department. The late Professor Bessey was a master in preparing such diagrams. His specialty was, as all know, the preparation of figures to show lines of descent and evolution of the groups of the plant world. Whether one could agree as to the derivations and progress of evolution represented by his diagrams or not did not detract from the fact that his figures helped greatly to portray the principles and the theories that he wished to emphasize. Thousands of his students were enabled to secure a much better idea as to what evolution really is from such methods even if they went no further in their study of biology than the Freshman course.

Figures or diagrams of this sort may be studied indefinitely and the longer they are examined the more illuminating they become until the average student is enabled to secure a complete and prop-

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THE HISTOLOGY AND PHYSIOLOGY OF THE PLANT

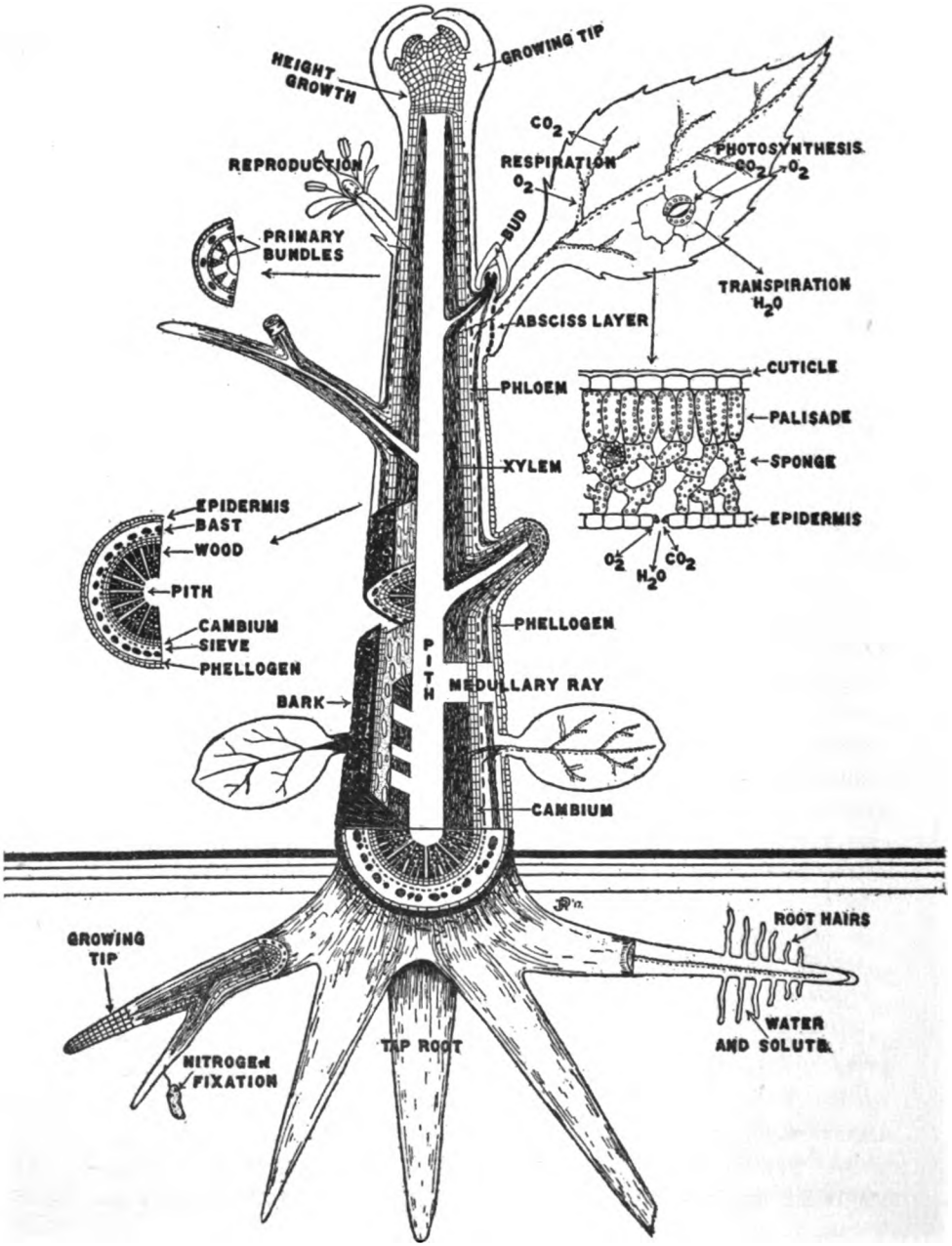

PLATE VI

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erly balanced conception of the chief features of the subject in question.

Such figures may be constructed in great detail and with much technical skill or they may be more or less crude and diagrammatic and still be of great value to the learner. With the information gained from careful studies of plant anatomy and plant processes the student should be able to fill in the missing details of a generalized drawing and to interpret properly the diagrammatic features of the figure. This being possible, a diagram of the kind submitted herewith should be exceedingly helpful and, indeed, illuminating. It was solely because of the success of these methods in this department that I was led to publish these brief notes and the diagram in the hope that they might be suggestive in some degree to my fellow workers in biological fields.

The figure as published here has been revised and redrawn from a similar figure published in 1914 by the writer in a laboratory manual of plant physiology. The older diagram was later enlarged and worked out in the form of a wall chart. The reader will understand, of course, as he looks at this diagram that the relative proportions of the various structures exhibited are not intended to be represented at all as they actually occur in the living plant. In fact the proportions are mostly so unnatural as to be grotesque and even misleading if the student or reader does not understand how to interpret them in the light of what has been said in the above paragraphs. He is supposed to have acquired this understanding in his courses of study dealing with the plant. The figure merely helps him to *see* the *interrelations* of the facts of histology and physiology graphically summarized in the features of the chart. The chart represents in a more or less diagrammatic manner an epitome of the great facts of histology and physiology. Many additional entries might be made upon the chart, but the danger is in so multiplying details that the real purpose of the sketch may be obscured behind the maze of unessential details. That would be a serious blunder in the use of this method of teaching or learning.

I believe that it is one of the chief duties of a teacher to epitomize very carefully his subject as fully as possible by whatever

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methods he may devise. In so far as he is successful in this practice so far will the students in his classes carry away with them a definite and compact and possibly useful body of knowledge upon the subject taught. Such a chart as this has been found very useful in such an epitome, and the method in general is successful in application.

RAYMOND J. POOL, PH. D.
The University of Nebraska.

A METHOD FOR MOUNTING ANATOMICAL PREPARATIONS FOR EXHIBITION

Oftentimes students in comparative anatomy make excellent preparations which are worthy of preservation. But this is not done as a rule because of the trouble in mounting. A glass strip of just the right size to fit into the exhibition jar must be found. The preparation is with difficulty tied by thread to the glass plate. If one could only "pin" into glass! The following method is suggested as a solution. A mixture of hard paraffin, beeswax and lampblack is melted up and poured into a paper box cover about the size needed had a glass plate been used. The mixture in the cover should be between ¼ and ½ inch deep. It should be allowed to cool somewhat and then on this bed the preparation should be placed and pressed down into it somewhat. A few small pins can easily be made to fasten it securely. When the matrix is cooled they can be clipped off on the back. Labels can also be easily attached to parts of the dissection. The entire cover can now be placed in cold water for a few minutes. When hardened the cover can be cut away with a knife—the paraffin background cut down to just the size that will fit into the glass jar—care being taken to make it fit in snugly. The jar can now be filled with formalin and the cover fastened on. The black background makes the objects stand out distinctly and the preparation never becomes loosened from its wax bed. The whole operation takes but a few minutes time.

G. G. SCOTT.

Department of Biology,
College of the City of New York.

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GREEN LIGHT FOR DEMONSTRATING LIVING CESTODE OVA

By projecting the light from a small arc through two screens, one of Picric Acid yellow and one of Methyl Blue blue, a green light was obtained which gave a peculiarly sharp definition to the details of living Cestode ova. The eggs were studied on a slide under either low or high power. This light has been used in connection with the demonstration of the eggs of *Dipylidium caninum, Tænia crassicollis* and *Hymenolepis nana.* The color screens were prepared by staining lantern slide plates, after dissolving out the silver with Hypo from the unexposed plate, in either a weak solution of Picric acid or Methyl Blue. In each case the film was stained until it showed a distinct color when washed in water.

University of Colorado. M. M. ELLIS.

A NEW METHOD OF STAINING TISSUES CONTAINING NERVES

Captain Sydney M. Cone, M. D. (J. Am. Med. Assn., Jan. 19, 1918) proposes the following method for nerves, which he says is excellent in bringing out axis cylinder and medullary sheaths, being approached only by Bielschowsky's Axis Cylinder stain. The latter requires days, and does not give contrast staining.

(1) Harden in 4% formaldehyde for 6 days; (2) carry thru graded alcohols to ether and absolute alcohol; (3) embed in celloidin and cut 10-20 microns, washing in water; (4) stain 15 minutes in carbol fuchsin, washing rapidly in water; (5) place in 1% osmic acid 5 minutes; (6) wash rapidly, and stain for 1 minute in 50% aqueous solution of safranin; (7) place in 1% acid alcohol 1 minute; and in 95% alcohol for 2 minutes; (8) place in absolute alcohol and clove oil alternately until sections appear deep pink and translucent; (9) place in xylene 2 minutes, and mount in Canada balsam.

Paraffin sections sometimes stain fairly well, but it is not safe, as xylene used to remove the paraffin before staining interferes with the process. The formaldehyde hardening is essential for the success of the stain.

[I wonder whether terpineol could not be used here, or bergamot, or naphtha, or even toluol. It would be a good thing if labora-

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tory workers would give the newer results for clearing agents that do not decolorize stains, used after imbedding in (a) celloidin or parlodion, or (b) paraffin.—V. A. LATHAM, Abstractor.]

FONTANA'S SPIROCHETE STAIN

Fontana's method of staining spirochetes involves the use of the following preparations:—1. *Fixing* fluid: acetic acid, 1 c.c.; formalin, 20 c.c.; distilled water, 100 c.c. 2. *Mordant:* tannic acid, 5 gm.; phenol solution (1 per cent), 100 c.c. 3. *Silver solution:* Prepare a 0.25 per cent solution of nitrate of silver, which may be done with sufficient accuracy by dissolving a small crystal in half a test tube of distilled water and adding just enough ammonia solution to cause a slight permanent turbidity. 4. Distilled water.

3. *Process:* Prepare the slide to be stained by spreading the material from the syphilitic lesion very thinly on a clean slide, allowing to dry spontaneously; fix by pouring on the fixing fluid, pouring it off after a few seconds. Renew immediately, and perform this process several times. The total duration of this stage should be *not less* than a minute. Wash well in distilled water, flood with the mordant, apply gentle heat until steam arises, and allow the process to continue for half a minute; wash thoroughly in distilled water (15 to 30 seconds), flood with the silver solution, again warm gently for half a minute, wash, blot and dry. Mount in balsam for permanent specimens. The spirochetes are stained jet black, and appear larger than when stained by ordinary methods. Cedar oil causes the spirochetes *to pale.* V. A. L.

SIMPLE METHOD OF CLEANING OLD USED SLIDES

Johnson (J. Am. Med. A., Dec. 8, 1917) suggests soaking slides indefinitely (24 hours at least) in full strength commercial (household) ammonia, followed by rinsing with water and wiping clean. Stained smears of all kinds, immersion oil, balsam mounts are equally well cleaned. The same supply may be used repeatedly if kept in tightly closed receptacle. V. A. L.

MENTHOL FOR NARCOTIZING

Don't forget that menthol is an effective reagent in narcotizing or anæsthetizing lower forms of life such as Rotifers, Infusoria, or even small crustacea. V. A. L.

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FRESH WATER BIOLOGY

Teachers in America have been eagerly awaiting this volume for several years. The knowledge of the plans of the editors and publishers, including as they have the collaboration of a large number of active biologists, has inevitably become wide spread. The need of such a work, planned on a scale that would be at once liberal and feasible, has been so genuine that it was assured in advance of a very wide use among teachers and working biologists.

The unanimous verdict will be that every reasonable expectation has been met by Professors Ward and Whipple and their helpers, in spite of the fact that the delays inevitable in getting such a synthetic task before the public will cause many of the contributors themselves to feel the need of revising their work by the time of its first appearance. All teachers of Biology, all advanced students of any group, all amateurs who use the microscope on living things, will find "Fresh Water Biology" a necessary part of their equipment.

The volume is, in many ways, very close to the kind of work which has long been fostered and advanced by the American Microscopical Society and its Transactions. An organized interest in limnological work was manifest as early as 1899, at which meeting a Limnological Commission, consisting of Professors Birge, Eigenmann, Kofoid, Ward and Whipple, was appointed to "unify, extend, and stimulate limnological work in this country." The following year this Commission made a report which anticipated much of the ecological work done since with the fresh-water forms of this country, and unquestionably gave inspiration and impetus to the studies on which this book is based. While the volume cannot be listed among the annual *"Transactions"* of the Society, certain it is that much of the contributory work leading to this fine showing in American fresh-water Biology has been done by members of this Society and published in one form or another in its *Transactions.*

So close is this enterprize to what this Society has been encouraging in every possible way for many years, that the pages of the *Transactions* are now freely offered the editor and collaborators,

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pending new editions of the book, for such supplementary and revisional statements as may be necessary from time to time to keep the accounts and keys of the various groups up to date. Such a cooperative arrangement would contribute greatly both to the convenience of our membership and to the most effective use of this manual.

The work is much too compendious and condensed to allow an adequate statement even of its scope, much less to bring to our readers any of its specific contents. In general the material presented is to be classified under three headings: (1) General discussion of the conditions of life and of the effective study of organisms; (2) the biological conditions, method of collection, culture and preservation of the special groups; and (3) systematic keys, with descriptions and illustrations of the classes, orders, families, genera, and representative American species of the groups treated.

Under the first head may be included the introductory chapter by Professor Ward, the chapter on "Conditions of Existence" by Professor Shelford, on "Methods of Collecting and Photographing" by Professor Rieghard, and the concluding chapter by Professor Whipple on "Technical and Sanitary Problems," as related to fresh waters. The biological features of the special groups are treated at the beginning of the appropriate chapters. The chapters on Bacteria, Larger Aquatic Vegetation, and Aquatic Vertebrates are confined to this aspect, making no effort at systematic display.

The following experts furnish the systematic chapters: Edgar W. Olive, Blue-green Algæ; Julia W. Snow, Other Fresh Water Algæ; C. H. Edmondson, Amœboid Protozoa; H. W. Conn and C. H. Edmondson, Flagellate and Ciliate Protozoa; Edward Potts, The Sponges; Frank Smith, Hydra and Other Fresh Water Hydrozoa; Caroline E. Stringer, The Free-living Flatworms; Henry B. Ward, Parasitic Flatworms; Wesley R. Coe, The Nemerteans; N. A. Cobb, Free-living Nematodes; H. B. Ward, Parasitic Roundworms; H. S. Jennings, The Wheel Animalcules; H. B. Ward, Gastrotricha; Frank Smith, Aquatic Chætopods; J. Percy Moore, The Leeches; A. S. Pearse, The Fairy Shrimps; E. A. Birge, The

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Water Fleas; C. Dwight Marsh, Copepoda; R. W. Sharpe, Ostracoda; A. E. Ortman, Higher Crustaceans; R. H. Wolcott, The Water Mites; James G. Needham, Aquatic Insects; Charles B. Davenport, Moss Animalcules; Bryant Walker, The Mollusca.

Two devices in the arrangement of the systematic matter call for comment. The guide numbers in the artificial keys are arranged in accordance with a plan developed by Professors Forbes and Smith at the University of Illinois. Each guide line begins with a number. In addition to its own appropriate number which leads, there follows in parentheses the alternative number (or numbers) which indicates the contrasted line to which the seeker must go if that particular legend is not diagnostic. This is true both of the earlier and the later guide lines in a given series. If a given key line is acceptable the further guiding number is at the close of the line. The device thus gives a perfect system of cross references both forward and backward between categories of a given grade. This is unnecessary in brief keys; but where there are scores of intervening subordinate categories it is a great convenience. The name and description of a species, all the supplementary biological facts concerning it and the illustration are included in a solid panel between its own key line and the next. This gives a convenient compactness which is very satisfying.

The general impression which follows examination of the book is the perfectly enormous amount of material condensed into its somewhat more than 1000 pages. This means, of course, great brevity, and yet no one interested in these groups can feel that the interesting and important matter has been left out. To one whose studies are confined largely to a single group there must come a renewed and enlarged sense of the representative character of the fresh-water organisms. One has brought home to him also the vast incompleteness of our records of the American distribution of even the better known fresh-water species. It ought to be possible in connection with the extended use and further revision of such a work as this to get a better account of specific range in this country.

It seems ungenerous to mention slight imperfections where so much has been brought to our aid. However, the appearance

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of the chapters on Protozoa and Oligochætes is marred by the use of occasional cuts too heavy and opaque to give any true idea of the delicacy of the organisms. Figure 982 of *Chætogaster* is an example of this.

A list of important references, in no case purporting to be a complete bibliography, concludes each chapter. An adequate index, including important descriptive terms and all of the scientific names used in the keys, concludes the book.

Fresh Water Biology, by Henry B. Ward and G. C. Whipple, with a staff of Specialists collaborating. Pages ix and 1111, with 1547 text figures. John Wiley and Sons, New York and London, 1918. Price, $6.00.

AN INTRODUCTION TO THE HISTORY OF SCIENCE

Nothing which has come to the attention of the reviewer puts more convincingly the meaning of the history of science than the preface of this little book by Professor Libby. "The history of science has something to offer to the humblest intelligence. It is a means of imparting a knowledge of scientific facts and principles to unschooled minds.

"The history of science is an aid in scientific research. It places the student in the current of scientific thought, and gives him a clue to the purpose and necessity of the theories he is required to master. It presents science as the constant pursuit of truth rather than the formulation of truth long since revealed; it shows science as progressive rather than fixed, dynamic rather than static, a growth to which each may contribute.

"It is only by teaching the sciences in their historical development that the schools can be true to the two principles of modern education, that the sciences should occupy the foremost place in the curriculum and that the individual mind in its evolution should rehearse the history of civilization.

"The history of science should be given larger place than at present in general history. History of science studies the past for the sake of the future. It is a story of continuous progress. It is rich in biographical material. It shows the sciences in their interrelations, and saves the student from narrowness and premature specialization. It affords a unique approach to the study

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of philosophy. It gives an interest in the applications of knowledge, offers a clue to the complex civilization of the present, and renders the mind hospitable to new discoveries and inventions.

"The history of science is hostile to the spirit of caste. It reveals men of all grades of intelligence and of all social ranks co-operating in the cause of human progress. It is a basis of intellectual and social homogeneity.

"Science is international,—English, Germans, French, Italians, Russians—all nations—contributing to advance the general interests. [The teaching of it] cannot fail to enhance in the breast of every young man or woman faith in human progress and good will to all mankind."

In method, this introduction takes up certain great scientific relations and applications, and treats these largely in connection with the personality of the men who have contributed their solutions. Some of the graphic chapter headings will carry the suggestion of method and of content:—1. Science and Practical Needs —Egypt and Babylonia; 2. Influence of Abstract Thought—Greece: Aristotle; 3. Scientific Theory Subordinated to Application—Rome: Vitruvius; 4. The Continunity of Science—the Medieval Church and the Arabs; 6. Scientific Method; 7. Science as measurement; 8. Cooperation in Science; 9. Science and the Struggle for Liberty; 10. Interaction of all the Sciences; 11. Science and Religion; 12. Reign of Law; 14. Scientific Prediction; 16. Science and War; 17. Science and Invention; 19. The Scientific Imagination; 20. Science and Democratic Culture.

The presentation is simple, direct, vivid, untechnical, and well suited to the intelligent reader with general interests.

An Introduction to the History of Science, by Walter Libby. Illustrated; 288 pages. Houghton, Mifflin Company, Boston, 1917. Price, $1.50, postpaid.

A SHORT HISTORY OF SCIENCE

Evidently the stay and the work of M. Sarton in this country is helping create an atmosphere in which we may prophesy an extension of interest in the history of science. In this atmosphere our own American teachers, who have been doing something in this field for their students, are being encouraged to bring their work to the

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more general audience. All this is very much worth while and will stimulate the giving of similar courses in many of our schools and colleges, both to culture the general student and to unify the scientific consciousness of the student of science.

The book under review is by Professors Sedgwick and Tyler and embodies very largely the well known course of lectures on the subject begun by the senior author in Massachusetts Institute of Technology more than twenty-five years ago. The purpose is expressed by the authors thus,—"To furnish a broad general perspective of the evolution of science, to broaden and deepen the range of the students' interests, and to encourage the practise of discriminating scientific reading, . . . by furnishing the student and the general reader with a concise account of the origin of that scientific knowledge and that scientific method which, especially within the last century, have come to have so important a share in shaping the conditions and directing the activities of human life."

The general treatment is broadly chronological and geographic, —following the origin and rise of the wonderfully varied civilizations of the near-Mediterranean peoples and their distinctive marks upon the progress of knowledge, of its applications, and of the method and spirit which its right pursuit demands of its followers. The chapter headings indicate this phase of the treatment: Early Civilizations; Early Mathematical Science in Babylonia and Egypt; Beginnings of Science; Science in the Golden Age of Greece; Greek Science in Alexandria; Decline of Alexandrian Science; The Roman World,—The Dark Ages; Hindu and Arabian Science; Progress to 1450 A. D.; A New Astronomy and the Beginnings of Modern Natural Science; Mathematics and Mechanics in the Sixteenth Century; Natural and Physical Science in the Seventeenth Century; Beginnings of Modern Mathematical Science; Science in the Eighteenth Century; Modern Tendencies in Mathematical Science; Advances in Science in the Nineteenth Century.

Within these general headings, further analysis and presentation are based upon a combination of biography, the rise and solution of problems, and the discovery of the principles which have proved significant and fruitful. Topics like the following raise the expectations of the reader and indicate the emphasis: Primitive

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Interpretations of Nature; Astrology; Primitive Counting and Geometry; Mathematics in Egypt; the Calendar and Measurements of Time; Greek Mathematics; Pythagoras; Beginnings of Rational Medicine; the Hippocrates; the Sophists; Circle Measurements; Aristotle; Euclid; Archimedes; Earth Measurements; Beginnings of Human Anatomy; Mathematics and Astronomy at Alexandria; Ptolemy; Hindu Astronomy; Arabian Contributions to Mathematics and Astronomy; Renaissance and Sciences; Alchemy; the Compass; Clocks; Textiles; Printing; the New Astronomy,—Copernicus, Tycho Brahe, Kepler, Galileo; Medicine and Chemistry, Anatomy; Vesalius; Higher Algebraic Equations and Symbolic Algebra; Gregorian Calendar; Harvey and Blood Circulation; Studies of the Atmosphere, Barometer, gases; Phlogiston; Beginnings of Chemistry; Bacon and Descartes; and thus on to the great wave of mathematical and natural science discoveries of the eighteenth and nineteenth centuries which cannot even be enumerated here.

The book is enlivened thruout by appropriate quotations from the men who did the work and from appreciative commentators on that work. In a series of appendices are more lengthy documents, —as, the oath of Hippocrates, Dedications by Copernicus and Harvey, Gallileo before the Inquisition, and the like. Appendix I enumerates and discusses briefly some leading inventions of the last two centuries.

The volume closes with a table of the important dates in the history of science and of civilization, a brief list of reference books, and an index. Each chapter closes with a list of references.

The book is attractively made up and printed.

A Short History of Science, by Sedgwick and Tyler. Illustrated, 474 pages. The Macmillan Co., New York, 1917. Price, $3.50.

BIOCHEMICAL CATALYSTS IN LIFE AND INDUSTRY

This volume discusses only the proteolytic enzymes, being the second volume by the author on enzymes and their uses. A preliminary chapter discusses the nature of the transformations that take place in the living cell, the inorganic catalysts, the biochemical catalysts, the theories as to their mode of operation, and a classification of proteolytic enzymes based on the number of molecules of

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water they are capable of fixing in a molecule of albumin. Following Schutzenberger's conception of the structure of the polypeptide molecule, the author presents a very attractive and cogent statement of the mechanism of progressive hydrolysis of these molecules under ferment action.

The general discussion proceeds under these heads:—The *Coagulating Catalysts,*—thrombin, myosinase, and rennet; *Pepsin; Trypsin,* both pancreatic and from various animal and vegetable sources; *Erepsins,* including those secreted in the intestines, the poorly defined peptolytic enzymes which act on so-called peptones, nucleases which transform the phosphoric nucleo-proteins, arginase, and a small group of creatin-destroying catalysts; and the *Amidases,* the group of enzymes which aid in the final decomposition of the amino-acids,—the last stages of the reduction of the protein molecule before assimilation or excretion.

The statement of the nature, origin, mode of isolation, properties, and physiological role of these vital substances is extremely lucid, and meets the need of the general biologist who has not the opportunity to keep abreast with the more technical aspects of this department of biochemistry.

Most general readers will be especially attracted to Part VI, which deals with the applications of these organized catalysts to medicine and industry, together with the grounds upon which such applications are possible. The author traces the use and abuses of pepsin in therapeutics, and progress made in standardizing tests of its efficiency both as to dissolving and in actual peptonizing power. Reference is made to peptones, both peptic and pancreatic, offered as an easily assimilable diet for greatly debilitated patients. Similar preparations are used in making culture broths in bacteriological laboratories.

In a similar way diagnosis of stomach states is made by analysis of the gastric contents at different stages of test meals, with a view to obtaining the amount of chemical change, the acidity, and the enzymic contents. The author holds that the disrepute into which this determination has fallen is due to poor methods of application rather than to any fault of the principle itself.

In preservation and use of grains and flours native proteolytic

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enzymes, and those produced by micro-organisms on the surface of the grains or placed in the flour purposely, bring changes that must be considered. So in brewing and in grain distillation, these biological catalysts play an essential role. The same processes are seen in the milk ferments and in the ripening of cheeses. In the latter some of the enzymes are native to the milk, some are produced by micro-organisms, and rennet is added artificially.

There is an interesting discussion of the relation of the proteolytic milk ferments to intestinal putrefaction. The writer himself has done work with the Bulgarian ferment, and his views as to the cause of the benevolent intestinal action of the various clotted milks are contrasted with those of Metchnikoff and others.

Other topics discussed are:—putrefaction, enzymes operative in tanning, biocatalysts of the soil, assimilation of atmospheric nitrogen, fertilizers, recovery of nitrogenous wastes, and artificial nitrogenous foods.

As the outcome, largely, of his own experiments the author sums up his conclusions in respect to the last item thus:—"It appears that there is ample proof that the organism draws all its nitrogenous constituents from the hydrolysis of proteins. These may result either from the actual process of digestion, or from artificial means, like the action *in vitro* of proteolytic enzymes or the action of concentrated acids. In all events, these [artificially reduced nitrogen molecules] are directly assimilable substances and should be considered as food materials of great nutritive value.

In fact, it has been established that a mixture of amino-acids, containing qualitatively and quantitatively all the principal products of the complete hydrolysis of proteins, can replace the albuminoid foods, and as such maintain the animal organism in nitrogenous equilibrium." The writer is convinced that nutrition can ultimately be effected more economically and rationally by the substitution of some of these artificially produced nitrogenous foods for the complex natural ones, such as meat.

Each chapter is followed by a bibliography; and an index closes the book. The mechanical part is well done.

Biochemical Catalysts in Life and Industry, by Jean Effront. Translated by Samuel C. Prescott, 752 pages. John Wiley and Sons, New York, 1918. Price, $5.00, postpaid.

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PROCEEDINGS

of the American Microscopical Society

MINUTES OF THE PITTSBURG MEETING

The thirty-sixth annual meeting of the American Microscopical Society was held in affiliation with the A. A. A. S. at Pittsburg, Pa., Dec. 29, 1917.

In the absence of President Guyer, Vice-President Griffin acted as chairman.

The report of the Custodian was presented and was accepted, ordered printed and referred for audit to a committee consisting of Professor Griffin and any other Pittsburg members whom he might select.

The Treasurer's report for the years 1916 and 1917 was accepted and referred to an auditing committee consisting of Drs. Latham and McCalla of Chicago.

The Society approved the recommendation of the Treasurer that the fiscal year be regarded as extending from Dec. 1 to Nov. 30.

The following officers were duly nominated and elected for the constitutional periods: President, Professor L. E. Griffin, University of Pittsburg; First Vice-President, Dr. H. M. Whelpley, St. Louis; Second Vice-President, Professor C. O. Esterly, Occidental College; Secretary, T. W. Galloway, Beloit College (for two years); Custodian, Magnus Pflaum, Esq.

In connection with the re-election of Mr. Pflaum it was noted that he has been custodian for eighteen years continuously since the formation of the office, having been Treasurer for three years before. A cordial vote of appreciation was extended him for this long and efficient service.

Professor Max M. Ellis of University of Colorado and Professor J. E. Ackert of Kansas State Agricultural College were chosen as the elective members of the Executive Committee for 1918.

Professors Griffin and Galloway were appointed a committee to approve and print the minutes. Adjourned.

T. W. GALLOWAY, *Secretary.*

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SPENCER-TOLLES FUND

Custodian's Report for the Years 1916 and 1917

Amount reported in 1915		$4489.32
Jan. 1, 1916, Dividends	$ 134.67	
July 1, 1916, Dividends	138.69	
Dec. 6, 1916, Sale of Transactions	60.00	
Dec. 15, 1916, Sale of Transactions	60.00	
Dec. 31, 1916, Dividends	142.86	536.22
		$5025.54
July 1, 1917, Dividends	$ 150.75	
Dec. 31, 1917, Dividends	155.28	306.03
		$5331.57

GRAND TOTALS

All Contributions	$ 800.27	
All Sales of Transactions	878.38	
All Life Memberships	300.00	
All Interest and Dividends	3542.92	$5521.57

LESS

All Grants	$ 150.00	
All Dues on Life Members	40.00	190.00
		$5331.57

Life Members: (Robert Brown, dec'd); J. Stanford Brown; Seth Bunker Capp; Henry B. Duncanson; A. H. Elliott; John Hately.

Contributors of $50 and over: John Aspinwall; Iron City Microscopical Society; Magnus Pflaum; Troy Scientific Society.

MAGNUS PFLAUM, Custodian.

Pittsburgh, Pa., Dec. 29, 1917.

We, the undersigned committee, hereby certify that we have carefully examined the above account for the years 1916 and 1917 and found the same correct.

L. E. GRIFFIN, Chm. Auditing Committee.

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ANNUAL REPORT OF THE TREASURER OF THE AMERICAN MICROSCOPICAL SOCIETY

December 24, 1915, to December 24, 1916

RECEIPTS

Balance on hand from 1915	$ 476.65
Membership dues	532.10
Initiation fees	129.00
Subscribers	302.20
Sales of Transactions	150.40
Advertisers	184.90
Miscellaneous	2.68
Total Receipts	$1777.93

EXPENDITURES

Printing Transactions, volume 34, no. 4	$ 269.28
Printing Transactions, volume 35, nos. 1, 2, and 3	592.13
Plates for Transactions, volume 34, no. 4	8.91
Plates for Transactions, volume 35, nos. 1, 2, 3 and 4 (in part)	74.92
Advertising literature	37.00
Office expenses; stenography, supplies, etc.—	
Secretary	134.59
Treasurer	26.70
Postage and express—	
Secretary	109.78
Treasurer	31.90
Mailing Transactions	16.16
Spencer-Tolles Fund from sales of sets of Transactions to U. of Pa. and to Notre Dame U.	120.00
Purchase of partial sets of Transactions	45.50
Expenses of Secretary at Columbus meeting, 1915	31.65
Sundry	5.70
Balance on hand	273.71
	$1777.93

Respectfully submitted,

H. J. VAN CLEAVE, Treasurer.
Feb. 15, 1918.

We hereby certify this statement corresponds with the Treasurer's book.

V. A. LATHAM,
GEORGE EDWARD FELL,
Auditing Committee.

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ANNUAL REPORT OF THE TREASURER OF THE AMERICAN MICROSCOPICAL SOCIETY

For the year beginning Dec. 25, 1916, and ending Dec. 21, 1917

RECEIPTS

Balance on hand from 1916	$ 273.71
Membership dues	880.00
Initiation fees	42.00
Subscribers	119.80
Sale of Transactions	174.00
Advertisers	435.00
Partial payment for cuts	23.14
Total receipts	$1947.65

EXPENDITURES

Printing Transactions, volume 35, no. 4	$ 259.28
Printing Transactions, volume 36, nos. 1, 2, and 3	543.74
Plates for Transactions, volume 35, no. 4	29.64
Plates for Transactions, volume 36, nos. 1, 2, 3, 4	78.35
Postage and Express	75.42
Office expenses—	
Secretary	73.37
Treasurer	32.26
In part payment of Secretary's expenses to Pittsburg meeting	50.00
Binding set of Transactions for sale	20.00
Miscellaneous items	16.02
Balance on hand	770.57
	$1947.65

Respectfully submitted,

H. J. VAN CLEAVE, Treasurer.
Feby. 15, 1918.

We hereby certify this statement corresponds with the Treasurer's book.

V. A. LATHAM,
GEORGE EDWARD FELL,
Auditing Committee.
Feb. 12th, 1918.

I hereby certify that I have examined the vouchers and accounts of H. J. Van Cleave, Treasurer American Microscopical Society, for the period ending Dec. 21, 1917, and I find the balance of $770.57 as shown on the books to be correct.

F. S. JOHNSON, Public Accountant.

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GALLOWAY'S ZOOLOGY

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OF THE

AMERICAN MICROSCOPICAL SOCIETY

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T. W. GALLOWAY,
Secretary-Editor.

Beloit, Wisconsin.

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EDITED BY THE SECRETARY

T. W. GALLOWAY

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TRANSACTIONS
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American Microscopical Society
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THREE NEW SPECIES OF AMEBAS: AMOEBA BIGEMMA NOV. SPEC., PELOMYXA LENTISSIMA NOV. SPEC. AND P. SCHIEDTI NOV. SPEC.

ASA. A. SCHAEFFER

The classification of the amebas[1] is peculiar in that two methods of species determination are followed. The larger amebas are classified according to the characteristics possessed by the cytoplasm and the general character of the vegetative stage, while the smaller amebas are being described according to the method of nuclear division prevailing during reproduction. In the larger amebas a study of nuclear division is extremely difficult and for many species impossible, owing for one thing to our ignorance of culture methods by means of which these species could be raised in abundance. On the other hand the small amebas exhibit so little cytoplasmic differentiation that specific determinations on this basis seem impossible.

A specific determination is interesting, however, from at least two points of view. One is the viewpoint of establishing blood relationships of descent between the different species, or systematics proper. The other point of view is a purely practical one, i.e., quick identification. The physiologist or the experimentalist wants a quick and correct method for identifying the organism he is working with. It is obvious therefore that if an ameba possesses characteristic cytoplasmic differentiations which may be observed at any time, the ameba will come to be recognized by these characteristics rather than by a complicated series of nuclear events which occur only occasionally and are frequently made out only with difficulty. In short, as a means of identification, cytoplasmic char-

[1] The word ameba is used as a common name for naked rhizopods lacking internal skeletons such as *Amoeba, Pelomyxa, Protamoeba, Endamoeba, Nägleria, Hyalodiscus, Dinamoeba, Vahlkampfia*, etc.

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acters are preferable where they exist; where these do not exist, recourse may be had to the nuclear division process.

All the larger amebas possess cytoplasmic differentiations in sufficient number and conspicuousness to serve as a ready means of recognition. Many of these characters are subject to very slight variation as a result of changes in the environment. Individual isolation pedigrees carried on for upwards of a hundred linear generations together with many collateral lines under varying food conditions, showed that most of the cytoplasmic characters are hereditary and practically uninfluenced by what might be said to be the most common environmental changes (Schaeffer, Science, 1916, p. 468). Amebas are therefore in this respect like all other groups of animals and the method of classifying them according to cytoplasmic differentiations is therefore sound.

These considerations should convince especially our younger microscopists that the investigation of our larger amebas is not nearly as difficult or forbidding a field as might be imagined from the great amount of labor that has been expended on the study of the life histories and nuclear phenomena of some soil and parasitic amebas during the past decade. The fifty or so species of aquatic amebas thus far described represent beyond any question only a very small fraction of the number of species in existence, and this number of known species could probably be doubled within a few years by careful examination of our marshes and ponds.

AMOEBA BIGEMMA NOV. SPEC.

Diagnosis. Size in locomotion, 100 to 300 microns long. Form very changeable. Pseudopods, numerous, tapering, blunt, never with sharp points. Surface smooth, no fine folds or ridges. Endoplasm usually containing numerous small twin crystals; crystals attached to 'excretion spheres.' Movement rapid, about 125 microns per minute. Nucleus single, spherical or slightly ovoid, about 12 microns in diameter; chromatin in small masses clumped loosely together in the center of the nucleus in a nearly spherical mass about 6.5 microns in diameter. Contractile vacuoles small about 15 microns in diameter; numerous; no coalescence among them; systole slow. Endoplasm filled with small vacuoles. Food: flagellates, ciliates, diatoms, rhizopods, nematodes, vegetal tissue, etc.

This ameba, for which the specific name *bigemma* is proposed, resembles to some extent the figures and descriptions of Parona's *digitata*, Mereschkowsky's *angulata*, Gruber's *spumosa* and Penard's *vespertilio*. In fact I regarded it at first as the *angulata* of Mereschkowsky or the *vespertilio* of Penard, which it occurred to me might possibly be synony-

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mous. But after further study of the characters of this ameba I began to suspect that my earlier conclusions regarding its specific reference might be mistaken. I accordingly investigated the specific characters of this ameba in connection with some experimental work, under widely varying conditions for about three years, and compared my observations with the published reports of earlier investigators of amebas with the result that I am unable to confirm the specific descriptions by any of the authors named from this ameba.

In the first place, Mereschkowsky's ('79) description is extremely vague (pp. 203-204) Mereschkowsky says the plasm of angulata is transparent, that it contains two kinds of grains: a few large ones and numerous small ones. About three contractile vacuoles and a small round nucleus are present. Few, "am ende zugespitzten (doch nicht wie bei A. *filifera* mit welcher *A. angulata* viel Aehnlichkeit hat) und die gestalt dicker, breiter Kegel habenden, vom Körper ausgehenden Pseudopodien characteristisch. diam. 0.0235." Movements very rapid. The figure illustrating this description is very crude. With the exception of size, this description as far as it goes might apply to a number of species of amebas. The size, 23.5 microns, is very much smaller than that of *bigemma*.

Parona's description of *A. digitata* (1883. Essai d'une Protistologie de la Sardaigne. Arch. des Science physiques et naturelles. T. 10. Troisième periode. p. 225-243. 1 plate) is somewhat more definite than Mereschkowsky's of *angulata*. *A. digitata* possesses a very granular endoplasm, a rounded and conspicuous nucleus, a large contractile vacuole, "pseudopods longs, conique et aigus," pseudopods always in small number. Movement is rather slow. Size, 63 microns (p. 228). The only three characters which may be considered distinctive are the size, the conical and pointed (the figure shows needle points) pseudopods, and the number of contractile vacuoles. None of these characters however are found in *bigemma*. Parona makes no mention of vacuoles in the endoplasm, which, if he had seen a *bigemma*, he could not have helped seeing, since these vacuoles are quite as conspicuous as the nucleus. There can be little question, I think, that Parona described another ameba than *bigemma* under the name *digitata*.

Leidy('79) figures several amebas resembling *bigemma*, *vespertilio* and *digitata* more or less closely, but he regarded them all as varieties of *proteus*, or as forms of uncertain specific reference.

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In his description of *A. spumosa* Gruber is no more explicit than Mereschkowsky in the instance mentioned. *Spumosa* has broad flat pseudopods, a vesicular nucleus, an endoplasm filled with vacuoles, no granules, a size of 25 microns, according to Gruber. As emended by Penard ('02) *spumosa* possesses these characteristics: A length of from fifty to one hundred and twenty-five microns; form resembling the foot of a goose, with very fine longitudinal lines on the surface; numerous vacuoles; contractile vacuole as much as thirty microns in diameter; a great many bicuspid granules of very small size in the endoplasm; nucleus deformable like that of *A. limax*; a compact nucleolus with a narrow margin of nuclear sap between it and the nuclear membrane. Althought I am inclined to accept the emendation of Penard because of its making for greater definiteness and stability in this difficult genus, yet it appears to me that instead of really emending or elaborating Gruber's description, he actually describes a new and different species. It is evident that another ameba than *bigemma* was under observation by both Gruber and Penard when these authors wrote their descriptions of *A. spumosa.*

Penard's description of *A. verspertilio* (1902, Faune Rhizopodique du bassin du Leman. Geneva, pp. 714) is as follows: size, about seventy microns length; pseudopods have always a conical form, their extremities being usually sharp pointed although the point may be slightly rounded occasionally for a moment[2]; posterior end sticky, dragging debris along as it moves forward; a profusion of extremely small green grains, and sometimes large excretion grains, in the endoplasm; a sperical nucleus with a compact nucleolus which is often covered with fine points; contractile vesicles one, two or three; almost always a considerable number of vacuoles appearing and disappearing as if they were contractile in the endoplasm (pp. 92-95). Penard's figures (p. 94) resemble in a general way the figures of *bigemma*, but in the important points such as the number and character of vacuoles, the shape of the pseudopod extremities, the relative diameters of the nuclear membrane and the nucleus, the character of the "nucleolus," stickiness of the posterior end, inclusions, etc., there is little resemblance between *vespertilio* and *bigemma.*

[2] . . . les pseudopodes ont toujours une forme conique, anguleuse; leur extermite est en principe acérée; mais parfois la pointe peut s'arrondir pour un instant (p. 93). His figures all show sharp needle points on the extremities of the pseudopods.

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The *Amoeba bigemma* is of medium size, being usually from 100 to 300 microns long in locomotion. Occasionally the size is very much greater. In several old cultures the amebas frequently arrived at a length of 500 microns in locomotion. As a rule, the average size in new cultures is about 150 microns.

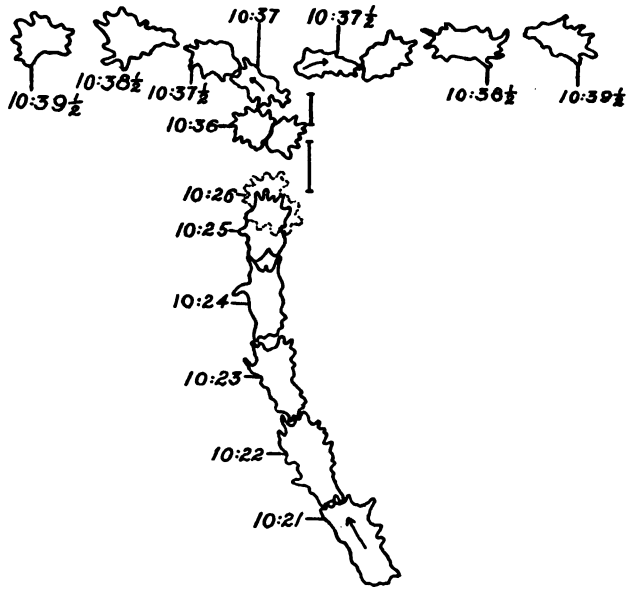

Fig. 1. Camera lucida drawing of an *A. bigemma* in locomotion during the process of fission. The figures indicate the time in hours and minutes. The drawings from 10:37½ on were moved up from their true position the length of the vertical line below; likewise for all drawings from 10:36 on. Note slowing down in the rate of locomotion as the fission crisis was approached, and the gradual increase of speed after division.

Although the general shape of this ameba is subject to very great variation, yet the various transformations are very characteristic. Perhaps the most characteristic feature of these transformations is the tongue like pseudopods, usually short, sometimes very long, which are continually being thrown out at the anterior end and on the free surface during locomotion (Pl. VII, fig. 2). These projections are frequently more or less conical, though more often perhaps they have somewhat the shape

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of a hart's tongue in outline. These projections are not pseudopods in the same sense that the projections in *dubia* or in *proteus* are pseudopods. In the latter species a pseudopod gives direction to locomotion, that is, the whole ameba frequently flows into a pseudopod. In *bigemma*, however, the whole ameba almost never, if at all, flows into a pseudopod; but the tips of the pseudopods advance at about the same rate of speed as the anterior end of the ameba as a whole advances. The pseudopods are, therefore, to be regarded rather as "toes" on the single pseudopod of which this ameba usually consists. During active locomotion the general shape of the body is most frequently triangular, with the broad base advancing, and more or less compressed. When the animal is disturbed it assumes a spherical shape. When suspended in the water for some time the ameba assumes a shape closely resembling that of the rayed state of a *radiosa*. From a central spherical mass of about thirty or forty microns diameter, long, slender, tapering pseudopods are thrown out which are of a much more permanent character than the pseudopods thrown out during locomotion. These projections are sometimes perfectly straight and of equal size and disposed opposite to each other on the spherical mass. More often however they are curved and of irregular shape and size. Their number is usually from six to eight. All the pseudopods formed during locomotion or while suspended are blunt, very definitely blunt. Of all the thousands of individuals which I have examined in all the different stages and cultural conditions, I have never seen any but definitely obtuse pseudopods. The photographs of live amebas, figs. 4 and 5 indicate the degree of obtuseness characteristic of the pseudopods of this species (Pl. VII).

The streaming of protoplasm during locomotion presents some points of interest. One does not observe the endoplasm flowing slowly in a definite direction in this species as one may in *proteus* or *dubia*, for example, but the streaming is jerky and irregular. The endoplasm seems to drain from the sides and posterior end toward the anterior end against numerous obstructions which often give way. Thus there are developed momentary counter or cross currents and eddies. The peculiar character of the streaming is due to several causes. In the first place the numerous pseudopods are formed and retracted without definite reference to the direction of locomotion. Then again the upper surface of the ameba is not level but extremely irregular; one observes a confusion of high ridges and deep depressions thrown together without

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observable order. From the depressions more or less permanent pillars of stiffened ectoplasm pass down to the lower surface obstructing the flow of endoplasm. When these pillars give way, as they frequently do, the bottom of the depression is suddenly pushed upward as if the endoplasm were under considerable internal pressure. Another cause of the irregularity of endoplasmic streaming is due to the fact that the anterior end does not advance steadily over the whole front, but by a series of waves here and there. Such a wave consists usually of a web of ectoplasm flowing out between and connecting two or more of the pseudopods; then the web halts momentarily while the pseudopods push out again or while new pseudopods form.

The rear end of the ameba usually plays little part in locomotion in a special way; usually the rear end is smooth and free from any pseudopodial projections. Occasionally, however, a long thin flat pseudopod may be rapidly thrown out near the posterior end which fastens itself to the substratum so well that considerable force is required to dislodge it (fig. 2). The ameba sometimes becomes stretched out to several times its usual length before the pseudopod is pulled loose. This behavior also results often in another phase of movement that I have much more frequently observed in this ameba than in any other, viz., some of the endoplasm begins to flow toward the posterior end until the ameba is cut almost in two in the middle. The connection is so thin that one looks every second for the connecting strand to break, but it does not break. Sooner or later one or the other portion of the endoplasm flows back and the whole mass again becomes unified in streaming.

The rate of movement is rapid, being about 125 microns a minute. Although the anterior end advances very unevenly and irregularly, there are, nevertheless, long segments in the path of an ameba moving on a plane surface that are straight. There is present in this ameba, therefore, the same tendency to keep on moving in the direction in which it started to move as there is in *proteus* (Schaeffer, '12, '18; this point will also be discussed at length in a paper soon to be published).

There is present in this ameba a layer of very thin protoplasm on the outside of the ectoplasm, as usually defined, which moves forward over the ameba in the same direction as that in which the ameba moves, but at a more rapid rate. This is clearly shown by the forward movement over the ameba of small particles which cling to this layer of protoplasm (Schaeffer, '17).

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During locomotion there is usually a broad zone of clear ectoplasm at the anterior end. Not only are the smaller pseudopods in this region free from granules but the intervening spaces also consist of ectoplasm.

The nucleus is easily seen. Unless obscured by food masses photomicrographs of the living amebas usually show it (fig. 4.). It is spherical or very slightly ovoid (fig. 3). In an ameba of about 200 microns length the nucleus is about twelve microns in diameter. The chromatin consists of very small ovoid granules collected together in the centre of the nucleus in a slightly irregular oval mass of about six or seven microns diameter. The color of the chromatin is a pale bluish yellow green. Between the chromatin mass and the nuclear membrane, which is perfectly transparent, there is a zone of clear nuclear sap. The nucleus is single, though occasionally an ameba is found with two nuclei, which statement is true of course for practically all amebas. The nucleus is deformable though it is not often that one observes striking deformations as it is swept along by the endoplasm.

In one culture of large amebas of this species the nuclei were about twenty-eight microns in diameter, and the chromatin mass of irregularly spherical shape was about fourteen microns in diameter. When these amebas were slightly squeezed under the cover glass one or two masses of mostly perfectly homogeneous pale bluish yellow green material was pressed out from the chromatin mass. There were present here and there large spheres of denser material of the same color as the homogeneous masses. Both the granular mass and the homogeneous masses rounded themselves up and collected near the centre of the nucleus. The material making up the masses seemed to be of the same sort, though I did not employ staining methods to determine whether the interior of the granular chromatin mass was really chromatin or some other substance. It is, however, interesting to know that at least some of the material inside the chromatin mass is not granular while the outside material is.

There seem to be three kinds of vacuoles in this ameba in so far as their functions are concerned. The endoplasm contains scattered about in it everywhere numerous (100 or more) small clear vacuoles of various sizes, mostly under ten microns in diameter, which may be called *permanent* vacuoles. What the function of these vacuoles is, or what conditions are necessary to their origin, remains unknown. That all of these may become contractile is hardly possible, unless they retain their identity in the ameba's body for many hours.

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The second kind of vacuoles are the *contractile* vacuoles. These have the same general appearance as the permanent vacuoles, excepting that they are more refractive to light. These vacuoles arrive at a diameter of about fifteen microns before they contract. It is very seldom that a larger size is attained. The diastole of these vacuoles is rather slow. The systole is also very slow, occupying from two to six seconds. There are a number of contractile vacuoles present at one time. Under favorable conditions as many as four may be observed to be in the process of diastole at one time. The general appearance of the later stages of a contractile vacuole, that is, higher refractive index of its contents, possibly indicates that these vacuoles are different from the permanent vacuoles from their beginning.

The third kind of vacuole, which may be called the *fecal* vacuoles, are not frequently met with in amebas. These are large spherical vacuoles containing in proportion to their size a very small amount of fecal matter. These may reach a diameter of twenty or twenty-five microns and in occasional large specimens a diameter of from forty to sixty microns. I have not been able to ascertain whether the vacuole originates around the fecal matter or whether after the vacuole is partly formed the fecal matter is voided into it. Since however, the vacuole usually becomes very large, it is evident that the later stages of the increase in size is due to the contained solid matter. The systole of these vacuoles is very slow. The liquid contents is first expelled and then after a pause the solid matter is thrown out in the way common to amebas generally.

None of these different kinds of vacuoles seem to grow by coalescence; at least from extended observation with this point in view I have never seen a single case of coalescence, although vacuoles frequently remain in close contact for a minute or longer.

Another very interesting element in the endoplasm is the crystals. These are usually very numerous and conspicuous, ranging in size from one and one-half to two and one-half microns in length. The general shape is like that of an hour glass or dumb-bell. They seem to be formed of two bicuspid crystals attached to each other by their apices (figs. 2, 3). Under ordinary light they appear dark gray in color; but in polarized light they show up very brightly, and then their twin structure becomes very evident. This is the only ameba known in which such a twin structure of crystal formation is found. The polariscope shows an

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unmistakable twin structure, however, in only about half the cultures I have so far investigated. In the others the two points of light are joined by a bar of light so that one sees a band of light not constricted in the middle. It may be inferred, however, that in these cultures the earlier stages of crystal formation are also on the twin pattern. It has been found that the character as well as the amount of food influences the size and to some extent the character of the crystals formed. Perhaps the most striking thing about the crystals in this ameba is the fact that they are always attached to the so-called excretion spheres when these are present, as they nearly always are (fig. 3). There is never but one crystal attached to a sphere. The size of the sphere bears no relation to the size of the crystal, the spheres being in some cases just barely visible, while the crystals may be two microns long. When the the spheres are small the crystals are always attached to them at their middle. The sphere and crystal bear a remarkable resemblance to a fish embryo lying on its egg yolk, and they form interesting objects for observation as they tumble along in the streaming endoplasm. Occasional twin crystals are observed apparently free from attachment to spheres, but it is possible that the spheres are extremely minute in such cases, too small to be seen. The excretion spheres rarely exceed a diameter of three microns. In some cultures a few bicuspid crystals with irregular sides are observed occurring singly. The maximum size of these is about two and one-half microns. Occasionally also two twin crystals are found crossed, (fig. 3). Altogether, the crystals form the most definite specific character of this ameba, and the presence of such crystals attached to spheres in an ameba may be regarded as definitely proving its specific identity.

This species is a voracious feeder. Flagellates, shelled rhizopods ciliates, rotifers, nematodes, diatoms, etc., and especially bits of vegetal tissues and masses of bacteria, are readily devoured. The body is frequently stuffed with food.

This ameba is one of the hardiest known to me. I have kept numerous and continuous cultures, after being well established, for several years without much difficulty. The species is subject to very little variation excepting size. In nature this species must be considered rare, though it is found frequently where large masses of vegetation are undergoing decay in quiet water.

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PELOMYXA LENTISSIMA NOV. SPEC.

Diagnosis. Length in locomotion, 75 to 125 microns. Body usually very much compressed and applied closely to substratum. Changeable in shape, general outline oval with few pseudopods. Quiescent stage with numerous pseudopods of various shapes. Color of body brownish; of protoplasm, pale bluish. Uroid of fine or large root like projections. Rate of movement very slow, from 1 to 2 microns per minute. Nucleus spherical, about 14 microns in diameter. Chromatin in a spherical layer of granules about 11 microns in diameter, with spherical body about 2.5 microns in the centre of the nucleus. Two nuclei frequently present. Contractile vacuoles numerous, 40 or more; maximum size about 10 microns; systole sudden; diastole very slow. Numerous or few small irregular crystalline masses present. Numerous bacterial rods of about 4 microns length present. Only very few refractive (starch) bodies present.

This pelomyxa is readily recognized by its small size and its very slow rate of locomotion (Pl. VIII, fig. 6). It is, in fact, much the slowest moving pelomyxa thus far reported, and I, therefore, propose the specific name of *lentissima* for this species.

I have found this organism in large numbers in old cultures from the marshes of Lonsdale on several occasions. But on account of its small size and its habit of flattening itself out on and sticking close to the surface on which it moves, it more readily escapes detection than other amebas of the same size. The color of this species is a dull brownish, somewhat like that of *P. belevskii*, but not so deep a shade, owing to its smaller size. This color is, of course, due to the contained food materials and the indigestible remains of food objects. It seems to be a habit of this and other pelomyxas to carry for a long time indigestible materials from food objects before excreting them, if indeed some of this material is ever excreted while the animal is in the vegetative stage. The color of the protoplasm is of a bluish violet tint.

This pelomyxa as distinguished from the other species, flattens itself out and sticks very close to the surface during locomotion. At such a time it is thickest in the centre and gradually becomes thinner as the periphery is approached. Around the entire animal there is a clear zone of protoplasm which is hyaline and very thin and of which the exact outer limit is very difficult to see. Pseudopods are continually being extended and retracted from the entire periphery of the animal except from the posterior end. These pseudopods are of clear protoplasm except for a small number of very pale bluish granules which are frequently found at their bases. The pseudopods are broad or narrow and always blunt. They do not usually determine the direction of locomotion.

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The posterior end terminates in a uroid or group of root-like pseudopodial projections attaching the organism to the substrate (fig. 6). They play a part in locomotion but just what their function is has not been carefully determined. It is certain, however, that the uroid is not necessary to locomotion to the same extent as in *P. schiedti.*

Locomotion in this form is so extremely slow that it is difficult to tell just how it is accomplished. Movement occurs at the rate of from one to two microns a minute, so that it takes from 30 minutes to an hour and a half to move the distance of its own length, or three and one-half months to creep around a baseball. There is a slight but continual and irregular movement of the endoplasm of an oscillatory sort, which, together with slight changes of body shape and the retraction and projection of pseudopods, masks the definite forward movement of the pelomyxa. Figure 9 shows that this movement undoubtedly exists. But under ordinary circumstances it is impossible to detect definite and continuous forward streaming of the endoplasm. It seems as if forward movement was the sum of all the separate local streamings.

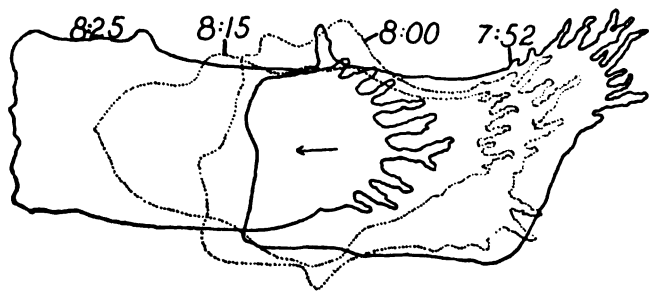

Fig. 9. Camera lucida sketch of a moving *P. lentissima* to show rate of, and form changes during, locomotion.

When suspended in the water or when loosened from the substratum this ameba is remarkable for the number of pseudopods which it throws out from all sides (fig. 7). This habit may indeed be regarded as a distinguishing characteristic of this species while in this stage. The pseudopods are usually more or less conical in shape though they always have blunt ends. The majority are of simple form, others are branched,

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while some are of very odd shapes. Their length is variable, the maximum being about 50 microns. As might be expected from their rate of locomotion, the transition from a spherical shape in the suspended stage to the locomotive stage consumes much time. As the organism again begins to move, a broad wave of clear ectoplasm appears at some point near the substrate. The wave slowly enlarges as the pseudopods are withdrawn, and gradually the locomotive form reappears.

The nucleus of this species presents several points of interest. In the first place there are usually two. In perhaps three out of four individuals examined two nuclei were found. In no case did I see more than two. It seems that in this species it is the normal condition for a considerable interval of time to supervene between division of the nucleus and the division of the organism. The two processes do not seem to be directly dependent on each other. In the case of most other amebas cell division must closely follow nuclear division, or else in the majority of cases it will become impossible for cell division to take place and the animal in consequence dies (Schaeffer, '16). So that what is normal in division sequences for *Pelomyxa lentissima* is pathologic for *Amoeba proteus*, for example. A *P. lentissima* with two nuclei may be looked upon as an individual whose cytoplasm has not yet divided.

Another point of interest with regard to the nucleus of this species is that a central body is clearly observable in the living condition. The composition of this body has not been investigated. The diameter of this body is from two to two and one-half microns. Its appearance is similar to that of the chromatin granules.

The general appearance of the nucleus is somewhat like that of *P. belevskii*. It is spherical, about fourteen microns in diameter, and contains a spherical layer of chromatin granules of about eleven microns diameter. Not very much can be said about the physical character of the nuclear membrane owing to the very slight movement of the endoplasm. The nuclei are usually found somewhere near the centre of the animal.

The contractile vacuoles are numerous. In one individual there were at least sixty, but whether all were contractile or not could not be determined. The average maximum size of these contractile vacuoles is about ten microns, though many contract when only five microns or so in diameter. Occasionally one may reach fifteen microns before contraction. Consonant with the slow rate of locomotion is that of enlarge-

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ment of the vacuoles. A vacuole of five or eight microns diameter may take fifteen minutes or longer to enlarge to ten microns, followed by contraction. The systole is, however, relatively rapid, occurring usually in about one second.

The spectroscope shows some very irregular small crystalline masses in the endoplasm sometimes numerous, usually comparatively few. Of "refractive bodies" (starch grains) there are only a few small ones present. Other inclusions in the ectoplasm are the bacterial rods, distinctive of the genus, reaching a length usually of four microns, very rarely of eleven microns; and the very numerous brownish masses of all sizes and shapes so common in several species of pelomyxas. In addition to these bodies there are also numerous very small greenish blue granules found in the endoplasm.

Besides an occasional diatom or shelled rhizopod, I have found no recognizable food bodies in this species.

PELOMYXA SCHIEDTI NOV. SPEC.

Diagnosis. Length in locomotion about 75 microns. Usual shape ovoidal. Color, brownish olive green, almost opaque. Pseudopods very rarely formed. Protoplasm fluid. Movement by eruptive waves of endoplasm partly reflected back along the sides. Rate of movement about 95 microns per minute. Nucleus sometimes single, usually double; spherical, about 7 microns in diameter. Chromatin granules in the form of a hollow sphere immediately under the nuclear membrane or at a slight distance from it. Contractile vacuoles small, numerous; maximum size about 4 microns; diastole rapid; systole instantaneous. Starch grains very numerous, irregular in shape, olive green in color, maximum size about 6 microns. Numerous bacterial rods present of 3 to 4 microns length. Small uroid always present during locomotion.

This species, the smallest of the pelomyxas has been found on several occasions in large numbers in old cultures of material brought from the marshes of Lonsdale (Pl. VIII, fig. 8). Because of its dark color and rapid rate of locomotion it at once attracts attention. Although three or four of my cultures were very rich in this form, it must, nevertheless, be classed as a rare species. The environment must be of a very special kind apparently in order that it may develop in numbers. It remained in my richest culture for about four weeks from the time it was first discovered. I propose for this pelomyxa the specific name *schiedti* in honor of my friend Professor R. C. Schiedt.

Under low magnification this organism appears quite black excepting at a few small places along the sides where the color is temporarily grayish owing to the accidental presence of but few of the starch grains so

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abundant in this ameba. The posterior end also usually is light gray. Under high powers however, the color is of a dull brownish olive green, due to the brownish endoplasmic inclusions and the numerous starch grains. The protoplasm is bluish green.

This species passes through very slight transformations in shape during locomotion or at other times (fig. 10). Its general shape during locomotion is ovoidal with the anterior end broad, while the posterior end is narrow. Never at any time does the organism flatten itself out to any extent on the substratum. The details of the process of locomotion have not been observed carefully, but the following details may be mentioned. Owing partly perhaps to the fluid nature of the protoplasm, no pseudopods are formed for the purpose of locomotion, but broad eruptive waves of endoplasm break out somewhere near the anterior end into which then flows a part of the animal's endoplasm. These waves are usually partly reflected back along the sides of the animal, leaving a more or less clear space at the farthest point reached by the reflected wave. Occasionally for a short period the pelomyxa may also advance by endoplasmic streaming as is commonly observed in amebas generally, but the larger part of its path is negotiated by eruptive waves as described.

Another important factor in locomotion is the uroid. The animal is not attached to the substratum anywhere except at the uroid. This is readily observed when they are taken up with a capillary pipette. The anterior part of the body is readily displaced by slight currents in the water but the posterior end is not affected in this way. It seems that the thin pseudopodium like projections of which the uroid is formed are for the purpose of holding the organism in place and at the same time allow it to move forward. But just how this is done could not readily be determined owing to the fact that these uroidal projections are very small and very transparent. It has been possible to determine however, that the projections may be thrown out very rapidly, almost instantaneously, so that it is possible that new projections are continually being formed as old ones are being retracted. The alternative view is that the uroidal projections are dragged along over the substratum attaching themselves temporarily and locally as they pass over the substratum. But whatever maybe the exact rôle the uroid plays in locomotion, it is evident that the organism is prevented from rolling over by reason of its attachment to the substratum.

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Although there is, as has been stated, considerable uncertainty in the direction which the waves of endoplasm take at the anterior end, the path of the organism may nevertheless, because of the activity of its prehensile uroid, be straight for a considerable distance. In a sense therefore the guiding agency in locomotion is located at the posterior end of the ameba.

The rate of locomotion in *schiedti* is rapid, being about 95 microns per minute (fig. 10).

The nucleus of this species presents nothing unusual. Its shape is spherical. The chromatin occurs in rather large masses arranged in the shape of a hollow sphere immediately underneath the nuclear membrane or at some distance from it. Usually two nuclei are present as in the case of *lentissima*. The binucleate condition therefore represents an intermediate stage between nuclear division and cell division. This stage in these two species (*schiedti* and *lentissima*) is much longer than in most typically uninucleate unicellulars, so that the binucleate stage is much more common. These pelomyxas are therefore to be looked upon not as binucleate organisms, but as typically uninucleate. The size of the nucleus is about 7 microns. Owing to the numerous endoplasmic inclusions, the nuclei are difficult to see in the living condition.

The contractile vacuoles are made out only with the greatest difficulty in normal individuals. They are seen only in the small clear areas which are observed occasionally along the sides during locomotion. With very attentive examination the vacuoles may then be seen. The maximum size of the vacuoles is about four microns. Nothing very definite can be said about their number which is certainly not less than ten, but is probably very much greater. The diastole is rather rapid. The systole is practically instantaneous, almost as rapid as the bursting of a bubble on the surface of water. There is a readily observed characteristic rush of protoplasm from the immediate vicinity to the place where the vacuole has just burst, which may be taken advantage of to locate a bursting vacuole without actually seeing the vacuole. In this way it is possible to determine that there are several systoles a minute in different parts of the animal. Doubtless the fluidity of the endoplasm is in some way connected with the small size and sudden contraction of the vacuoles.

This species is full of what are probably glycogen grains (Stolc, A., 1900. Zeit. f. wiss. Zool. Bd. 68). Their color is a shade of olive green.

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The shapes of the bodies are varied, mostly irregular, angular with rounded corners and edges. The maximum size commonly met with is about six microns. Most of them are only two or three microns long. They are not evenly distributed throughout the body, but there seems to be a tendency for them to collect very near the surface in what is called the ectoplasm. In focussing along the edge one observes a serrated outline, the teeth being represented by the protruding starch grains. I presume that a layer of protoplasm at all times covers these bodies when lying at the surface, though one cannot observe such a layer in the living organism.

Besides the starch bodies there are found considerable numbers of very small spherical bodies of a bluish green color. These are met with in nearly all amebas, but of their nature nothing is definitely known.

The bacterial rods, the presence of which characterizes the genus *Pelomyxa*, are found in considerable numbers in *schiedti*. The length of these rods is about three or four microns.

The number of brownish colored inclusions which are so commonly found in pelomyxas, is small in this species. Sometimes only two or three masses of appreciable size are found. Very little food has been observed in the bodies of these animals. Occasionally a diatom or a flagellate was seen, but in the great majority of individuals no recognizable food objects were found.

When the cultures began to die out, the glycogen bodies began to disappear gradually. In the last few surviving individuals almost no glycogen grains could be seen. The organisms were very pale yellowish and sluggish. Numerous large permanent vacuoles appeared. The nuclei also changed in appearance. The chromatin receded further from the nuclear membrane and collected itself in much larger but fewer granules than normally. From these observations we may conclude that the starch grains are reserve food stores as has been shown by Stolc to be the case in *P. palustris*, and that the cultures died out chiefly because of lack of food.

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EXPLANATION OF PLATES

PLATE VII

Fig. 2. Photograph from water color drawing of *bigemma* in characteristic attitude during locomotion. C, nuclear chromatin mass; C-S, crystals attached to spheres; F, food mass; M, nuclear membrane; V vacuoles.

Fig. 3. Photograph from water color drawing of nucleus and excretion spheres and crystals of *bigemma*. CR, chromatin granules of nucleus; C, twin crystals attattached to excretion spheres; M, nuclear membrane; S, spheres; Occasionally two twin crystals are attached to each other as shown.

Fig. 4. Photomicrograph from unretouched negative of several live *bigemmas* among diatoms and arcellas. The nucleus, N, is faintly shown in two of them. The arcellas measured 58 microns in diameter.

Fig. 5. Photomicrograph from unretouched negative of several live *bigemmas* in characteristic attitudes during locomotion. Note especially the blunt character of the ends of the pseudopods. Same magnification as figure 4.

PLATE VIII

Fig. 6. Photograph from water color sketch of a *P. lentissima* in locomotion. N, nucleus; CV, contractile vacuole; U, uroid.

Fig. 7. Water color sketch of a quiescent stage of *lentissima*. Note the numerous and bizarre shaped pseudopods.

Fig. 8. Water color sketch of *P. schiedli* in locomotion. CV, contractile, vacuole; N, nuclei; U, uroid. Note the numerous irregularly shaped starch bodies.

Fig. 10. Camera lucida sketch of a moving *P. schiedli* showing the slight changes in body shape during locomotion.

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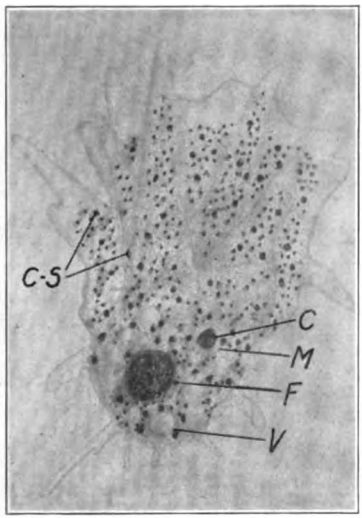

FIG. 2

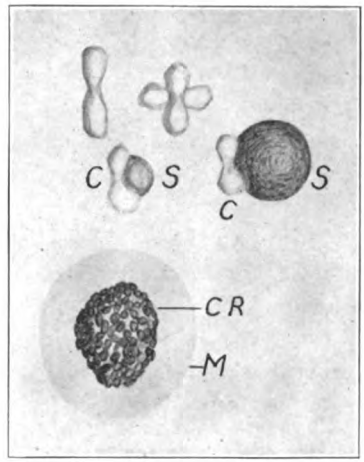

FIG. 3

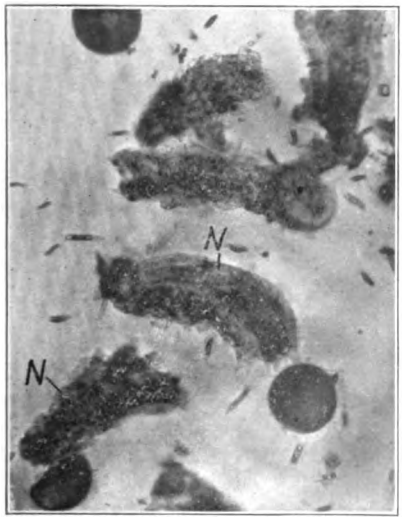

FIG. 4

FIG. 5

PLATE VII **SCHAEFFER**

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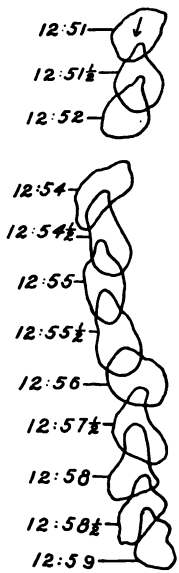
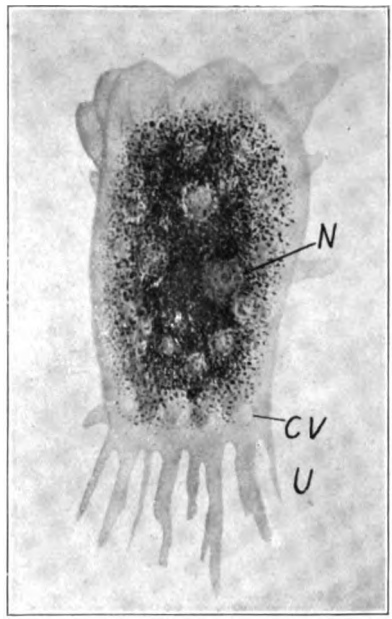


FIG. 10



FIG, 6

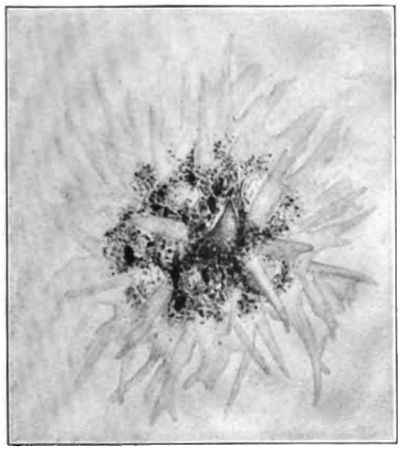


FIG. 7

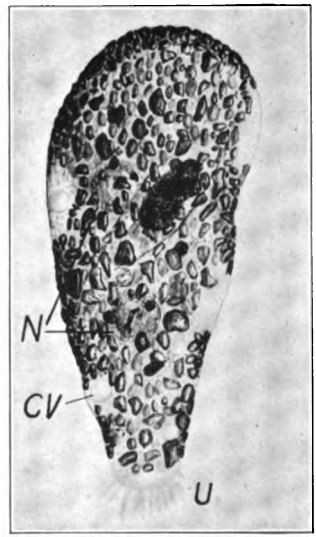


FIG. 8

PLATE VIII **SCHAEFFER**

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SPERMATOGENESIS OF THE DOG

BY

JULIAN Y. MALONE

The problem of spermatogenesis has been extensively worked on in insects and various other invertebrates. Recently several vertebrates have been shown to possess the so-called X-chromosome which is associated with sex-determination. Two hetero-chromosomes have been described in most of the mammals, and they probably correspond to the X- and Y-elements as described by Wilson ('12) in the Hemiptera. Different members of the latter class show a variation of these elements from forms which have only the X-element, thru those which have the X- and Y-element of unequal size, to those in which these elements are of equal size.

In the dog I find only one such element, the history of which I have tried to follow in this paper. The present study has been carried on at the suggestion of Professor M. F. Guyer, to whom I am especially indebted for kindly help and criticism. I wish also to acknowledge my obligation to Dr. Elizabeth A. Smith for valuable criticism.

MATERIALS AND METHODS

Thru the courtesy of the Department of Physiology of the University of Wisconsin, I was enabled to get the material in the living condition. Eleven of the animals from which the tissues were taken were mongrels; the twelfth was a thorobred bulldog. No especial differences were noted in the material except occasional minor discrepancies in the size of the cells.

At first samples from different regions of the living testes were placed directly into the fixing reagents in the preliminary preparations, but the best results were obtained when the tissues were allowed to stand for twenty minutes before being immersed in the fixative. As experienced by all other workers on mammals the great problem is to get the chromosomes to stand out as individual elements, the tendency being for them to mass-up before the fixative takes effect. For this reason the following fixatives: Gilson's, Carnoy's, Flemming's strong, Bouin's, Tower's, Herrmann's, Haner's modification of Flemming's strong, and Allen's modification of Bouin's, were tried out several times in an effort

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to overcome this difficulty. It was found that the latter reagent gave the best results. As urea in this solution presumably increases the rate of penetration of the fixative, the amount of urea was varied from one to five percent in order to determine the optimum concentration for this tissue. A four percent solution was found to give the most satisfactory results. The formula for this fixative is as follows: to 100 cc. of Bouin's fluid add slowly 1.5 gm. of chromic acid and 4 gm. of urea just before using. Heat to 37° C., add the fresh material and leave it in the fixative for one to two hours keeping the temperature up to 37° C. For good cytoplasmic fixation put the fresh material in the fixative at 60° C. allow to cool to 37° C. and keep at this temperature as before. The fixative is then replaced gradually by 70 percent alcohol until entirely removed. A convenient apparatus for this part of the technic is described by Allen ('16). The tissues are then dehydrated, placed in xylol or chloroform, xylol-paraffin and imbedded in paraffin. As the material fixed better after standing 20 minutes the experiment was tried to see if allowing the tissues to autolyze from one to six hours would improve the fixation. It was found that there was no perceptable difference in the appearance of the nucleus altho the cytoplasm showed evidence of being digested and the chromosomes showed a tendency to clump up more after the first hour of autolysis.

Flemming's strong and Haner's modification of Fleming's strong gave good nuclear figures but the chromosomes did not stand out very clearly. Carnoy's fixative gave good spindle figures but always distorted the cells.

The sections were cut 5 microns and 7 microns thick and were stained with: (1) Haidenhain's iron-haemotoxylin and counter-stained with eosin, Bordeaux red, acid fuschin, or orange G; (2) Saffranin-lichtgrun; (3) Saffranin-gentian violet; (4) Benda's mitochondrial method. The most satisfactory stain was the iron-haemotoxylin counter-stained with acid fuschin, altho the saffranin preparations were valuable in studying the resting stages, different phases of the growth period, and the shape and number of the chromosomes.

Smear preparations were fixed in Allen's modification of Bouin's fluid and stained with Haidenhain's iron-haemotoxylin and Benda's mitochondrial stain. These preparations gave as good results in all stages as was found in the sections and were of advantage in so far as the cells were isolated, thus removing the danger of any confusion due the surrounding cells or to part of the cell being cut away.

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Arrangement of the Germ Cells in the Testis

The main bulk of the testis is made up of the coiled seminiferous tubules which contain the germ cells. The seminiferous tubules are held together by connective tissue which contains a small number of interstitual cells, blood and lymph vessels. Practically all stages of development of the germ cells may be found in a single tubule. In general the spermatogonia and the Sertoli cells are at the periphery, then comes the primary spermatocytes, the secondary spermatocytes, the spermatids and the spermatozoa. There is no definite seriation of the stages in any one tubule, such as is seen for example, in the insect testes. Some tubules are filled practically with cells in the growth stages, others with spermatids and some with nothing but a few Sertoli cells and fibers left by the discharged spermatozoa.

It is thus apparant that the great problem in Mammalian spermatogenesis is to determine the seriation of the stages of development. The criterion employed was, mainly, resemblance to the stages in forms which had already been worked out. The size of the different cells and the number of chromosomes were the chief guides.

Spermatogenesis

Spermatogonia

The spermatogonia usually lie at the periphery of the tubules, but occasionally when rapid multiplication is taking place, they are found in the deeper portions. The spermatogonia are hard to distinguish from the Sertoli cells but usually they can be identified by the small amount of their cytoplasm. The nuclei of the Sertoli cells present the same general appearance as those of the resting spermatogonia and as far as I can determine are the same. According to some investigators they both have a common origin from the primordial germ cells (Hegner '14).

There are present in the resting spermatogonial nuclei from one to four large, deeply staining nucleoli which always fuse when activity commences. This process of fusion is seen best in the saffranin preparations, which show that these bodies approach each other but before coming in contact seem to be connected by thin threads of chromatin on each side. They then completely fuse forming an oval nucleolus (figs. 1-3). Even in the earliest periods when these bodies are well separated they seem to be connected by thin fibers or strands of chromatin. A number

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of interlacing linin threads along which small granules of chromatin are deposited radiate from the nucleolus (fig. 4). As activity commences in the nucleus there is a marked increase of chromatin granules about the nucleolus as though they were threading out from it (fig. 5). At the same time the amount of chromatin along the linin fibers increases until the linin is practically obscured from view. Undoubtedly some of this chromatin along the threads is from the nucleolus but the increase is so great that some must either be synthesized or is present in the resting nucleus in such a chemical structure that it does not react to the stains used The cytoplasm which is made up of short interlacing fibers contains in all stages a spermatosphere. This stains more intensely with cytoplasmic stains.

As development continues the separate threads of chromatin shorten and become thicker. These finally condense into the chromosomes of the spermatogonial metaphase, as shown in figs. 7 and 8. It appears as though a single thread does not become a single chromosome but that parts of threads condense about different "centers" which are connected to each other by the linin fibers. The nucleolus does not lose its identity thruout these stages but remains a large, dark body with threads radiating from it. It does, however, approach the chromosomes in size in the early prophase (fig. 7), but it soon becomes more darkly stained than they (fig. 8). Cells showing the nucleolus as in fig. 7 are very few hence it indicates that this stage is of very short duration. This so-called nucleolus is probably the X-chromosome as it can be traced as such throuout all the later stages by its staining reaction. The newly formed chromosomes now take up their usual position on the spindle as twenty-one oval-shaped bodies of which one is the X-chromosome. Information as to the origin of spindle fibers and of the centrosomes was not obtainable but they were very distinct in all cases. During the formation of the spindle, the nuclear wall disintegrates. The spermatogonial metaphase is probably of short duration as stages where none of the chromosome had started to divide, as in fig. 9, were very few. Counts were made from polar views (Fig. 10), and oblique side views of the metaphase spindles. In material obtained from a female foetus an attempt was made to determine the somatic count in tissues taken from the pancreas, liver, mesonephros, metanephros and kidney. A few clear spindles were secured from liver material in which 22 chromosomes appeared (fig. 69).

In cases where the autosomes had just started to divide (figs. 11, 13, 14), it was obvious that the X-chromosome was dividing ahead of them.

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In the middle anaphase (figs. 15 and 16) the X-chromosome can be seen still slightly ahead of the autosomes, which are now clumping together. The early telophase (fig. 17) shows the chromosomes clumped with a spermatosphere near each mass. The cell wall (fig. 18) constricts in the middle of the long axis and finally divides the cell into two complete daughter cells. The spindle fibers are still present but soon disappear leaving the cells with a mass of chromatin, and the spermatosphere imbedded in the cytoplasm. Thus the cells are ready for the growth period as primary spermatocytes.

Growth Period

Stage A-preleptotene (figs. 19 and 20). In this stage the cells from the spermatogonial telophase have the chromosomes clumped together in an irregular granular mass. The nuclear membrane has not yet formed. This stage possibly represents that described by Wilson ('12) in *Oncopeltus* and *Lygaeus* as an uncoiling of the individual chromosomes to form separate leptotene threads, but as the chromosomes are so massed the actual processes cannot be determined. A large black body which is present in this mass of chromatin retains its identity throughout the growth period and from its subsequent behavior will be called the X-chromosome. Fig. 20 shows the leptotene threads emerging from the chromatin mass.

Stage B-leptotene (fig. 18). The nuclear wall is now present and is seen to enclose several thin, beaded threads of chromatin and the X-chromosome. These leptotene threads do not form a continuous spireme but appear as independent threads in both the smear and section preparations. While the threads show no definite polarization such as Wenrick ('16) found in the *Phrynotettix magnus*, they form a network which makes them very hard to count. They have not been seen to exceed twenty in number which further indicates that they take their origin in the spermatogonial telophase chromosomes.

Stage C-synapsis and synizesis (fig. 22). The leptotene threads of stage B drift toward one pole of the nucleus where they condense into a mass. The parts of the nucleus not occupied by these threads is clear. This stage has been studied in several forms of mammals by von Hoff ('12) who concludes that synizesis is the result of the action of the fixative but as pointed out by Wilson ('12), Fasten ('14) and others it occurs in the living material and thus cannot be the result of the action

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of the fixative. In cases of poor fixation, however, the leptotene threads are so contracted that their individuality cannot be made out, while with proper fixation it is very clear that the threads do not lose their identity during this contraction. They appear to arrange themselves in pairs for they emerge from the mass in parallel strands.

Stage D-pachytene (fig. 23). The leptotene threads which paired up in the previous stage now fuse side by side; that is, undergo parasynapsis. This is conclusively shown in this figure as in many others where two leptotene threads can be seen fused at one end and separated at the other. The line of fusion of these threads is not obliterated until a much later stage. Thus the dog is another form which shows parasynapsis such as described by von Winniwarter ('09) Gregoire ('04), Schreiners ('04), Wilson ('12) Smith ('16), and others.

Stage E-diplotene (fig. 24). This stage is hard to distinguish from stage D except that all the leptotene threads have fused to form the thicker diplotene threads. In these the line of fusion of the leptotene threads cannot be seen except in well destained preparations. The ends of the threads appear thicker than the rest of the thread. This stage might be called the beaded stage for each thread has the appearance of a string of beads. They approximate the haploid number. It will be noted that thruout these stages the X-chromosome does not lose its identity and that the spermatosphere is present.

These diplotene threads now contract gradually into somewhat oval-shaped chromosomes. As this contraction progresses, linin threads connecting them appear. In the late prophase the nuclear wall breaks down and the chromosomes take their places upon the primary spermatocyte spindle. Since twenty-one chromosomes entered the primary spermatocyte from the spermatogonial division, the leptotene threads paired and the X-chromosome did not lose its identity, it is obvious that the spermatocyte autosomes must be bivalent; that is, each one is made up of two univalent chromosomes, and the X-chromosome is univalent. This material contained no indications that the leptotene threads twisted about each other to form chiasmas such as observed by Janssens ('09) and Smith ('16). Heterotypic tetrad figures in the late prophase stages are not apparent although preparations stained favorably with iron-haemotoxylin reveal a quadrapartite appearance of the bivalent chromosomes as tho they were preparing for the following maturation divisions.

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The actual growth period might be considered to be from stage C to E as there is very little change in volume up to the time of synizesis but from there on the increase is very marked. The diplotene stage probably lasts longer than any other as cells in this stage of development are found in large numbers in the majority of the tubules.

Reduction Division

When the primary spermatocytes are ready for division they reveal ten large bivalent chromosomes, and one large X-chromosome. As the X-chromosome lies, in the metaphase, in very close proximity to the autosomes it is often difficult to determine its shape. But it can be distinguished from the others as a longer, slightly curved body, (figs. 28, 29, 32, 35, 38, 39) similar to that noted by Guger ('12) and ('16). However, a curved body from the concave or convex side would appear as an oval body (fig. 30 and 34). Fig. 31, a polar view, presents clearly the reduced number of chromosomes. All the chromosomes of the equatorial plate were not usually in the same plane.

As division starts in the autosomes the X-element can be seen to pass slightly ahead of them to one pole. The dividing chromosomes are long and thin giving the appearance of overlapping each other (fig. 36 and 37). This division is probably the reduction division as the autosomes divide longitudinally and the X-chromosome passes unchanged to one pole. In fig. 41 there could be counted at one pole, ten ordinary chromosomes plus the accessory while only ten autosomes passed to the other pole. It will also be noted that the spermatosphere is still present.

Interkinesis

After the primary spermatocyte division no resting stage occurs. The secondary spermatocyte metaphase is formed by the rearrangement of the chromosomes present in the primary spermatocyte telophase.

In the late anaphase of the primary spermatocyte the centrioles with short spindle fibers between them and the chromosome masses (fig. 44) are apparent, indicating that the chromosomes do not reach the poles as in the other divisions. The division of the centrioles and the formation of the new astral system cannot be followed but from the appearances of such cells as figs. 46 and 47 one might infer that there is not a new astral system formed but that the remnants of the previous spindle are re-organized to form the secondary spermatocyte spindle. The

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chromatin mass is imbedded in a more or less clear space surrounded by cytoplasm.

As two types of cells enter this stage two kinds must result from their division, one with ten univalent autosomes, (fig. 49), and one with ten univalent autosomes plus the X-chromosome, (fig. 48). When the autosomes divide they pull apart in the center, the X-chromosome dividing slightly ahead of them and passing to each pole, fig. 52. In the telophase of this division the chromosomes are usually massed up but in such a figure as 54 approximately ten chromosomes plus the accessory can be distinguished passing to each pole whereas in others no trace of an accessory can be found, fig. 55. Thus two kinds of spermatids which will develop into mature sperm result from this division.

Spermiogenesis

The chromosomes of the second maturation division break up into an open reticulum composed of linin threads and chromatin granules. The nucleus thus goes into a resting condition which apparently lasts for some time. In approximately half of the nuclei there can be seen a definite round body, fig. 60, which possibly corresponds with the X-chromosome It can be seen in the nucleus after condensation of the chromatin has occurred and the nucleus has migrated to one side of the cell, fig. 62 and 63.

The spermatosphere, which takes the cytoplasmic stains, is present in all of the spermatids in the cytoplasm and either imbedded in it or closely associated with it can be seen the centrosome. In the same region of the cytoplasm, fig. 57, is found the idiozome or remnant of the previous spindle.

The centrosome and the idiozome are differentiated from one another by the fact that the centrosome remains in close apposition to the spermatosphere. It is a single, regular body while the idiozome is usually lobular and irregular. As the idiozome comes in contact with the nuclear wall an oval, clear space as described by Leplat ('10) in the cat appears between it and the nuclear wall (fig. 58). It is apparently caused by some repulsive force between the nuclear membrane and the idiozome for the nuclear wall is definitely depressed. The wall of the cavity opposite the nuclear wall is probably formed by material from the idiozome. The nuclear wall then gradually returns to its original position, fig. 60, the nucleus becoming longer than it is wide and the idiozome

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forming a cap over about two-thirds of its length. Later this cap becomes the acrosome of the mature sperm fitting closely over the anterior end of the head. There seems to be little change in the idiozome threads during this transformation but the dark mass at the tip disappears. In sections threads running between the Sertoli cells and the acrosome were usually noted but no indication of their origin could be found unless they come from the acrosome. Fig. 68 shows a tubule from which all of the sperm have been discharged and has nothing in it but a few Sertoli cells with these threads running to them.

While this development of the acrosome has been going on the spermatosphere and the centrosome have migrated to the opposite side of the nucleus. The spermatosphere becomes closely applied to the nuclear wall and the centrosome divides into an anterior and a posterior portion, fig. 61. At this stage the nucleus gets smaller and the chromatin material appears as an indefinite granular mass in which a dark body, possibly the remnant of the X-chromosome, is seen in approximately half of the cells, fig. 62. The nucleus migrates to the side of the cell toward the acrosome apparently carrying with it a definite amount of cytoplasm enclosed in a denser wall, fig. 62. The migration of the nucleus is usually toward the tubule wall. At the base of this cytoplasmic neck which is destined to become the sheath of the middle piece of the mature spermatozoa, is found the spermatosphere. It retains this position until the nucleus, now the sperm head, has broken thru the cell wall. In the "giant cells" (fig. 74) in which a number of spermatid nuclei are present in one cell, there is a spermatosphere associated with each nucleus.

The process of formation of the acrosome and middle piece is similar to that found by McGregor ('99) in *Amphiuma*, except that he finds part of the centrosphere or idiozome also going to form the middle piece.

After division of the centrosome the anterior one comes in contact with the nuclear wall causing a temporary depression in the latter and then flattens out into a disc between the walls of the cytoplasmic neck. This disc, which forms the end knob of the sperm, has extending back from it a thin filament which extends to the spermatosphere fig. 63. Attached to this filament by a fine stalk is the posterior centrosome. This condition differs from that described by Leplat ('10) in the cat and Wodsedalek ('13) in the pig, in that the posterior centrosome in these animals forms a ring which migrates along the axial filament and is cast off with the cytoplasm.

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As the sperm head breaks thru the cell wall the axial filament becomes much longer. The flattened head is composed of three regions of different staining reaction, fig. 64. The point of attachment of the acrosome corresponds with about the posterior margin of the anterior two-thirds of the head and the point of attachment of the cytoplasmic neck corresponds with the anterior margin of the posterior third of the head. Thus it appears that the difference in the density is due to the presence of these membranes and that the lighter middle portion is due to the absence of these membranes. Further evidence of this is seen in fig. 65 in which the acrosome is becoming applied to the nuclear wall. Here there are only two regions of different color. This observation may be carried to the mature sperm, fig. 67, which shows in a side view that there is a layer of dark staining material covering the posterior third of the head, which also appears darker than the rest of the head when viewed from above, fig. 66.

When the cells reach the stage shown in fig. 65 they become attached in groups to the Sertoli or nurse cells by long filaments. At this time the cytoplasm is cast off and the sperm continue to develop. The surrounding cytoplasm is found to disappear gradually and as the sperm do not leave the tubule until this is about complete, it is possible that they derive some of their nourishment from this bed of cytoplasm. In the Benda preparation there was found large and small globules of fatty substance in this cytoplasm, in the Sertoli cells and a few in the interstitual cells. These globules stain black with iron-haemotoxylin but do not stand out as well as in the Benda preparations.

In the final changes of the sperm the acrosome becomes closely applied to the sperm head and is no longer distinguishable. The cytoplasm of the middle piece contracts and slightly elongates to cover almost the anterior half of the sperm tail or axial filament. During this contraction the posterior centrosome breaks thru the cytoplasmic wall and is seen lying outside the middle piece (fig. 66). There is no evidence of the sperm head enlarging during this process as described by Wadsedalek ('13).

Thus the mature sperm is seen to consist of: a head formed from the entire nucleus of the spermatid, the cytoplasm of the middle piece from the spermatosphere and the axial filament together with the anterior and posterior centrosomes from the centrosomes. The spermatozoa now lose their attachment to the Sertoli cells and pass into the lumen of

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the tubule leaving behind the Sertoli cells with large bundles of threads attached to them as shown in fig. 68.

Further evidence of the dimorphism of the sperm was obtained by camera lucida measurements. Three hundred measurements were made at 2,000 magnification of mature sperm, obtained from the vas deferens. It was found that these sperm, selected at random from the preparation, could be grouped into two classes on the basis of size. The heads of one hundred and sixty-one at this magnification measured five to six millimeters when projected onto paper with a camera lucida while the balance of the three hundred measured seven to eight millimeters. This difference in size is probably due to the presence of the X-chromosome.

Summary

1. The five typical cells ordinarily found in spermatogenesis: spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, and sperm occur in the dog in an unseriated arrangement. These are enclosed in long, thin, winding tubules which are held together by connective tissue and interstitutal cells.

2. Large numbers of spermatogonia and Sertoli cells are present around the periphery of the tubules and may contain one or more large, deeply staining bodies or nucleoli. In case of the spermatogonia these nucleoli always fuse before activity is marked. This body is possibly associated with or is the X-chromosome.

3. The spermatogonia show twenty-one oval shaped chromosomes, the X-chromosome usually not being distinguishable until the early anaphase where it divides and passes to the poles slightly ahead of the autosomes.

4. Following the spermatogonial division the chromosomes weave out into separate leptotene threads, while the X-chromosome remains as a rounded or slightly oval dark-staining mass.

5. The leptotene threads undergo parasynapsis.

6. Eleven chromosomes appear in the primary spermatocyte, ten are bivalent autosomes and one the X-chromosome. The X-chromosome passes undivided to one pole while the autosomes divide by longitudinal splitting. Thus there are produced two kinds of secondary spermatocytes. This division is reductional.

7. There is no resting stage between the primary and secondary spermatocyte divisions, the chromosomes retaining their identity although they increase in size slightly.

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8. The two kinds of secondary spermatocytes upon division give rise to two kinds of spermatids, one with ten univalent autosomes and the other with ten univalent autosomes plus the X-chromosome. This dimorphism is further evidenced by the resting spermatids as approximately half of them show a large, deep staining body which is probably the X-chromosome.

9. The chromatin of the spermatid nucleus condenses into an indifferent mass, the nucleus contracts, becomes narrower and flattened. It passes to one pole of the cell, breaks thru the cell wall and leaves most of the cytoplasm of the cell behind. It then attaches itself to a Sertoli cell by a thin fiber and shapes up into a mature sperm.

10. During spermiogenesis the centrosome gives rise to the end knob, axial filament and the posterior centrosome; the sphere substance of the secondary spermatocyte division to the acrosome; and the spermatosphere to the sheath of the middle piece.

11. Measurements of mature sperm give a distinct bimodal curve, also indicating their dimorphism.

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EXPLANATION OF PLATES

All drawings were made at 1600 magnification with a camera lucida. The plates were reduced about one-fourth.

Plate IX

Figs. 1, 2 and 3. Resting spermatogonial nuclei.

Fig. 4. Resting spermatogonia.

Figs. 5 and 6. Spermatogonia in early stage of activity showing the increase in chromatin along the linin fibers.

Figs. 7 and 8. Spermatogonia showing the condensation of the chromatin to form the chromosomes of the metaphase. Fig. 8 is from a smear preparation.

Fig. 9. Side view of a spermatogonial metaphase.

Fig. 10. Polar view of a spermatogonial metaphase showing 21 chromosomes.

Figs. 11, 12, 13 and 14. Early spermatogonial anaphase showing the chromosomes starting to divide.

Figs. 15 and 16. Spermatogonial anaphase showing the X-chromosome dividing ahead of the autosomes.

Fig. 17. Early telophase of the spermatogonial division.

Fig. 18. Late telophase of the spermatogonial division.

Figs. 19 and 20. Preleptotene stage or beginning growth period. The dark staining compact mass is the body which later is seen to be the X-chromosome.

Fig. 21. Leptotene stage showing the X-chromosome.

Fig. 22. Synapsis and synizesis showing the drifting of the leptotene threads and their contraction to one side of the nucleus.

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Fig. 23. Pachytene stage showing the chromsomes pairing by parasynapsis.

Figs. 24 and 25. Diplotene stage showing the beaded appearance and the unfused threads.

Figs. 26 and 27. Late primary spermatocyte prophase showing the condensation of the diplotene threads to form the chromosomes.

Figs. 28, 29, 30, 32, 33 and 35. Side views of primary spermatocyte metaphase, figs. 28, 29, 32 and 35 showing the curved X-chromosome.

Fig. 31. Polar view of a primary spermatocyte metaphase showing ten autosomes and the X-chromosome.

Figs. 34, 36, and 37. Early primary spermatocyte anaphase showing longitudinal division of the autosomes.

Figs. 38 and 39. Parts of primary spermatocyte anaphases showing the curved X-chromosome going to one pole.

Figs. 40 and 41. Early primary spermatocyte telophase.

Figs. 42, 43 and 44. Late primary spermatocyte telophase.

Figs. 45, 46 and 47. Secondary spermatocyte prophase.

Figs. 48 and 50. Secondary spermatocyte metaphase showing ten autosomes plus the X-chromosome. Fig. 50 from a smear preparation.

Fig. 49. Secondary spermatocyte metaphase showing ten autosomes.

Fig. 51. Early secondary spermatocyte anaphase showing equational division. From a smear preparation.

Plate X

Figs. 52 and 53. Early secondary spermatocyte anaphase showing equational division.

Fig. 54. Secondary spermatocyte telophase showing approximately ten autosomes plus the X-chromosome at each pole.

Fig. 55. Same as fig. 54 except that the X-chromosome is not present.

Figs. 56 and 57. Spermatid showing the spermatosphere with the centrosome imbedded in it and the remnants of the secondary spermatocyte spindle (idiozome).

Figs. 58, 59, 60 and 61. Spermatids showing the formation of the acrosome from the idiozome, the migration of the spermatosphere plus the centrosome to the other side of the nucleus, and the division of the centrosome.

Figs. 62, 63 and 64. Spermatids showing the migration of the nucleus to one side of the cell, the formation of the middle piece, end knob, posterior centrosome and the axial filament.

Fig 65. Immature sperm which has cast off its cytoplasm except that destined to become the sheath of the middle piece and the fibre which seems to connect it with the Sertoli cell.

Fig. 66. Mature sperm viewed from the broad side.

Fig. 67. Mature sperm viewed from the side.

Fig. 68. Cross section of a tubule the sperm cells of which have all matured leaving only a few Sertoli cells and fibres.

Fig. 69. Polar view of a somatic cell of a female foetus showing 22 chromosomes.

Figs. 70 and 71. Anomalies showing heterogenic division.

Figs. 72 and 74. Giant cells showing more than one nucleus and their associated spermatospheres.

Fig. 73. Two spermatids side by side.

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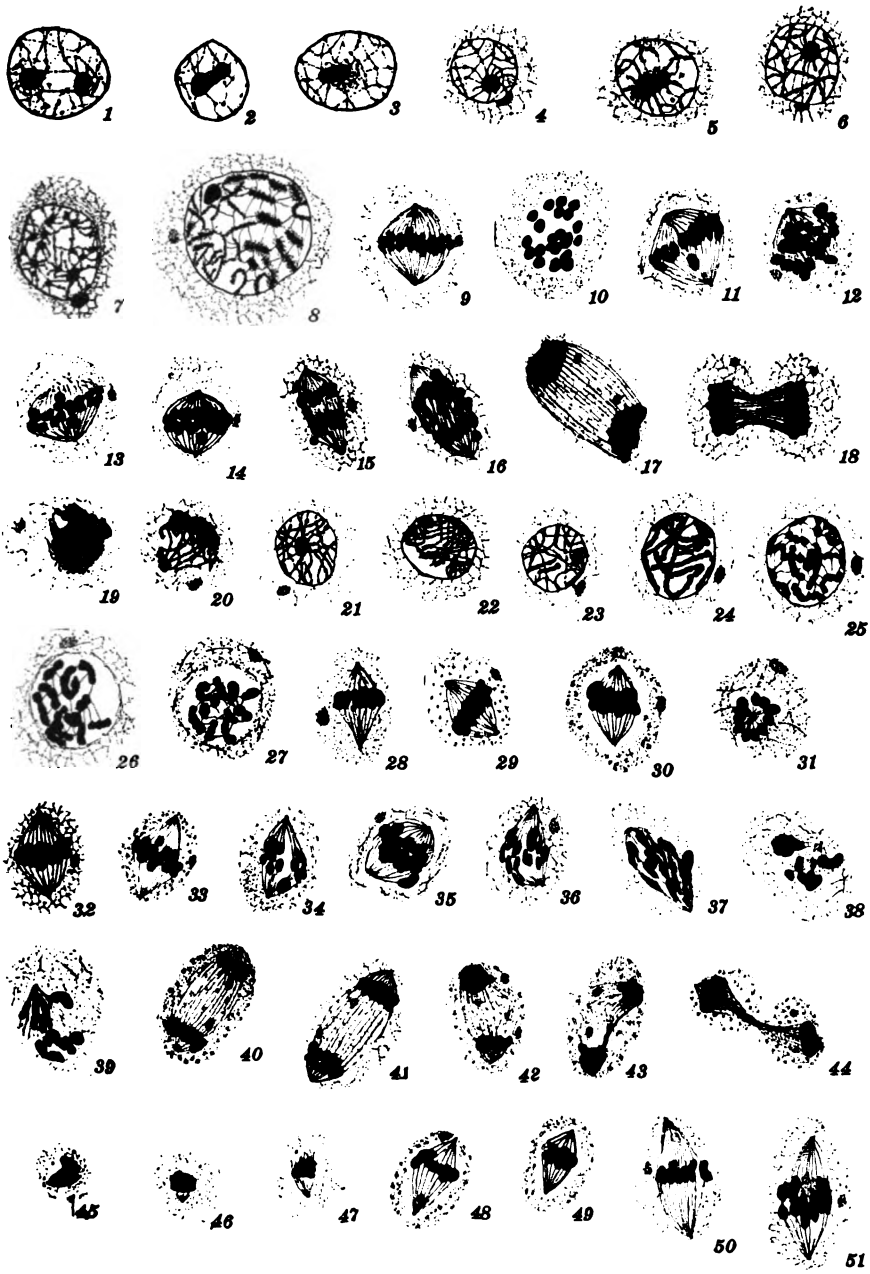

PLATE IX

MALONE

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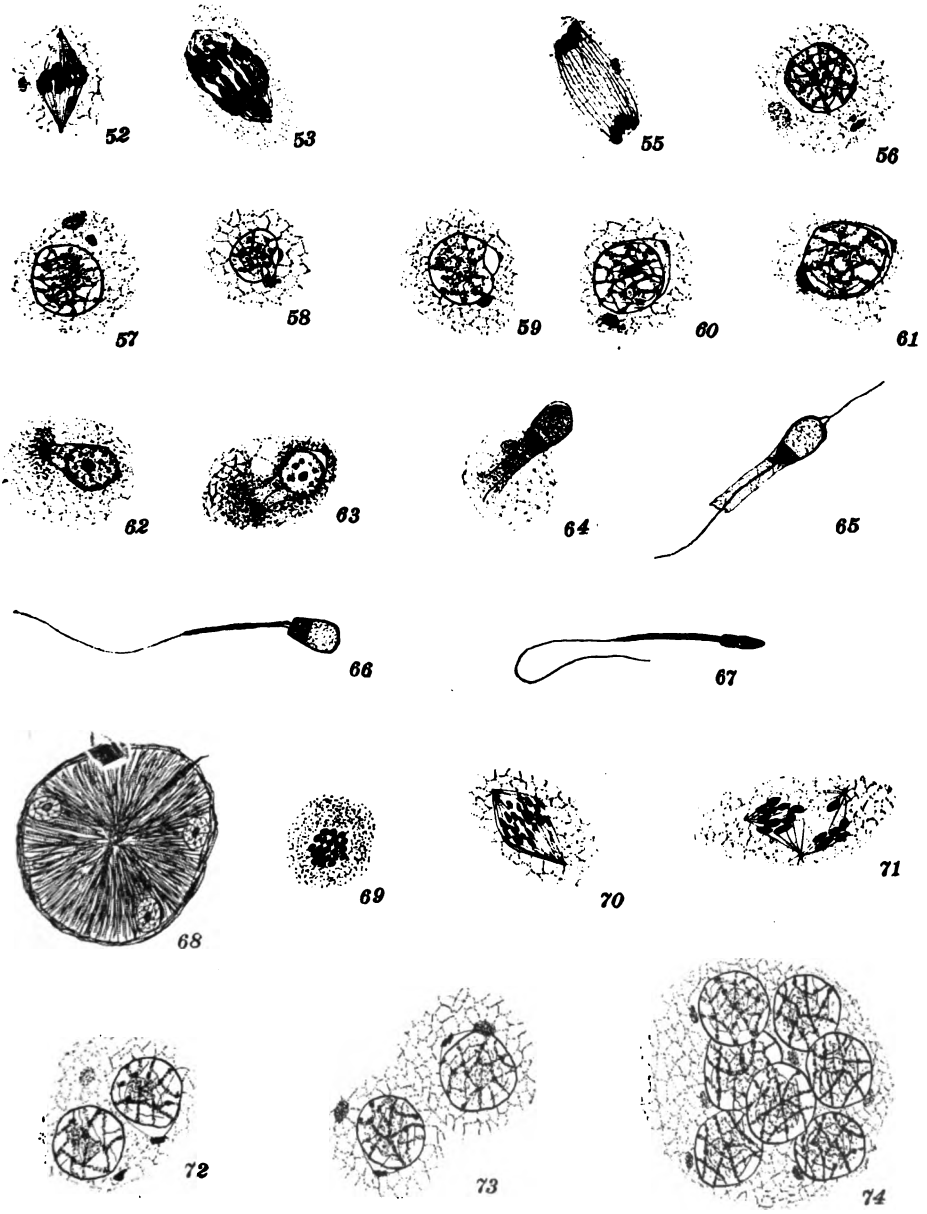

PLATE X

MALONE

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THIGMOTACTIC REACTIONS OF THE FRESH WATER TURBELLARIAN, *PHAGOCATA GRACILIS*, LEIDY

BY

BERNOL R. WEIMER

The Turbellaria have ever been popular subjects for experimentation, yet in looking over the literature, I find that very little has been done upon the thigmotactic reactions of these animals. Thinking that here was a problem that might yield some interesting results, I collected a number of the animals in Falling Run, Morgantown, West Virginia, and these were used in the following experiments. Five specimens were sent to Dr. H. S. Pratt of Haverford College, and were identified by him as Phagocata gracilis.

Six rocks of varying degrees of roughness were collected and cemented together with Portland cement in a circular glass dish 7 cm. high. The rocks were so arranged as to form a surface as nearly flat as possible. The outer walls of the dish were covered with heavy black paper in order that light could fall only vertically upon the surface of the rocks.

After allowing water, which was changed frequently, to stand in the dish for three weeks, some animals were placed in the dish. All the animals were in good condition, but those animals which came in contact with the cement between the rocks, secreted a slimy substance and died. The cement was then coated with paraffin, and at the same time a piece of glass of nearly the same area as the different rock surfaces, was waxed in.

After carefully washing the paraffin for two days in running water, some animals were again placed in the dish and after two days were alive and in good condition. Forty animals were then put into the dish which was placed beneath the skylight in a room so darkened that all the light came from this source. The surface of the rocks in the experiments was covered by water to an average depth of 1.5 cm. The temperature of the water varied from 21° -25° C.

In collecting the rocks used in the experiments, care was taken that the surface should have different degrees of roughness and that each rough surface should be as nearly uniform as possible. The rocks and the glass plate were each numbered with India ink. These numbers are used in the tables given, to designate the rock and, obviously, the surface. Rocks numbered 1, 2, 3, and 4 were of weathered sandstone and rock number 5 of weathered lime-stone.

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The surface of rock number one was composed of rounded quartz crystals of about the same coarseness as granulated sugar. Rock number two had a surface somewhat smoother than number one, the particles of sand were about as coarse as table salt. Rocks numbered four, three and six approached, in the order mentioned, rock number five, a smooth lime stone. Thus the rock surfaces varied in coarseness or roughness of surface from particles the size of granulated sugar to a comparatively smooth surface in the following order: one, two, four, three, six, and five. For a surface a grade smoother than the rocks described, there was the glass plate and the paraffin coating the cement. Practically the only surface difference noticed between the glass and the paraffin was, that the paraffin had a slightly greasy feeling.

The following explanation refers to tables 1 and 2. The total number of animals counted on a given surface during a set of observations was found and this number, divided by the number ofobservations in the set, gave the average observation for the set. This average observation for each set was added and the average of this total gave the average observation for all the sets. Since only one set of observations was taken in tables 3 and 4, the second average of the sets, was not necessary. The area of the paraffin and the vertical glass sides of the dish covered by water were averaged as one surface (para-glass in tables). This para-glass surface was used as the common unit area and from this the relative number of animals resting on a common unit surface, was computed. Since the paraffin and the vertical glass surface was so nearly alike, both were averaged as one. With this brief explanation, the tables given should not be difficult to understand.

The data set forth in table 1 was procured in the following way. The observations were taken at either ten or fifteen minute intervals and usually after each observation the animals were stimulated, i.e. caused to move from the place they were resting. No distinction was made between an animal moving across and one at rest on a surface. This probably accounts for the high averages for the various surfaces in table 1. The dish was frequently moved around to counteract any light reaction, tho the light apparently made no difference since it came vertically from above. A tendency to gather in groups was also noticed. These groups were usually found on the paraffin. The animals in these groups were always stimulated after an observation.

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TABLE 1

No. of lbs.	10	10	8	10	8	7	15	Total 68		
	av. set1	av. set2	av. set3	av. set4	av. set5	av. set6	av. set7	av. of all obs.	No. animals per unit surface	Total no. of animals counted
Rock										
1	1.70	1.80	2.75	0.70	2.00	4.30	1.60	2.13	25.53	145
2	2.40	1.20	1.50	0.40	2.55	3.66	0.46	1.73	15.65	108
3	2.10	3.40	2.70	1.10	2.44	2.50	1.05	2.19	18.79	249
4	0.90	4.80	8.25	0.50	2.20	1.16	1.90	2.81	34.59	192
5	2.30	2.40	1.66	0.80	1.60	2.30	1.90	1.85	15.17	126
6	1.40	1.20	1.40	1.30	1.70	3.60	1.26	1.66	17.66	113
Glass 7	1.70	0.90	1.00	0.70	1.30	2.10	1.20	1.27	16.25	86
Para-glass	32.70	28.50	25.12	34.50	28.30	30.30	31.86	30.18	30.18	2053

Observations showing number of animals seen on each surface with vertical illumination only. The time between sets varied from a day to a week. The surfaces in order from roughest to smoothest are as follows: one, two, four, three, six, five and glass.

From table 1 it is seen that when all the surfaces have been reduced to the same unit area, the three highest averages of animals resting, are as follows, rock four, 34.59, paraffin-glass, 30.18 and rock one 25.53. The other surfaces have much lower averages and do not vary much among themselves. One peculiar fact to be noticed is that rock one has the roughest surface, the glass-paraffin has the smoothest surface, and intermediate between these two is the surface of rock four. It would be well to remember that after each observation the animals were stimulated. Since so few animals were found resting, they were averaged with those moving.

The data in table 2 differs from that found in table 1, in that all the observations were taken in a photographic dark room. An electric light was used only long enough to record the observation. The observations were taken at intervals varying from 30 minutes to an hour and the animals moved from their resting position after each observation. As a result of the longer interval elapsing, most of the animals were found resting when the observation was recorded.

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TABLE 2

No. of obs.	4	6	6	4	Total 20		
	av. set 1	av. set 2	av. set 3	av. set 4	av. of all obs.	No. of animals per unit surface	Total no. of animals counted
Rock 1	0.00	1.50	1.60	0.75	.96	11.41	192
2	2.00	3.00	1.16	0.25	1.60	14.38	32.00
3	1.00	2.33	0.80	2.50	1.65	14.15	33.00
4	1.50	1.50	0.16	1.00	1.04	12.80	21.00
5	0.25	1.66	6.10	1.00	2.22	18.20	45.00
6	1.50	0.50	3.00	3.75	2.18	23.06	44.00
Glass 7	1.50	2.30	1.50	0.25	1.38	17.66	28.00
Para-glass	32.75	25.60	27.60	30.75	29.17	29.17	584

Observations taken in photographic dark room showing number of animals counted on each surface. The time between sets varied from a day to a week.

The surfaces in order from roughest to smoothest are as follows: one, two, four, three, six, five and glass.

The averages of these observations in table 2 show, that when all the surfaces have been reduced to the same unit area, the surfaces having the most animals resting upon them are the paraffin-glass, 29.17, and rock six, 23.06. Rock six has a comparatively smooth surface of weathered lime-stone. The averages for the other surfaces are about the same.

After conducting the first set of experiments, the results of which are found in tables 1 and 2, the glass was broken from around the cement block containing the rocks. The cement necessarily exposed was covered with a coating of bees wax. The whole block, surface downward, was then placed upon three glass supports, 2.5 centimeters high, in an enameled pan. The pan was then filled with water until it covered the surface of the rocks. This apparatus was first put in a photographic dark room, and observations taken each hour by lifting the rocks. The results were so decidedly negative that only a few observations were taken. See table 3. The animals not only did not rest upon the rock surfaces but were found scattered in average numbers over the entire

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bottom and sides of the pan. Only an average number was found on the bottom of the pan underneath the rocks.

TABLE 3

Surface	Observations					Av.	No. animals per unit surface	Total no. animals counted
Rock 1						Av.		
2	1					0.20	1.19	1
3		2				0.40	3.43	2
4								
5								
6								
Glass								
Para.								
Pan	39	38	40	40	40	39.90	39.90	237

One set of five observations taken in photographic dark room with the rocks inverted, showing the number of animals counted on each surface.

The surfaces in order from roughest to smoothest are as follows: one, two, four, three, six, five and glass.

TABLE 4

Surface	Observations									Av.	No. animal per unit surface	Total no. animals counted
Rock 1												
2									1	0.09	0.80	1
3												
4												
5									1	0.09	0.73	1
6												
Glass												
Para.					1		1			0.18	0.18	2
Pan	40	40	40	40	39	40	39	40	38	39.5	39.50	435

One set of nine observations taken with vertical illumination only and with rocks inverted, showing the number of animals counted on each surface.

The surfaces in order from roughest to smoothest are as follows: one, two, four, three, six, five and glass.

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The same apparatus was then placed underneath the skylight in a room so darkened that all the light came from this source. The same negative results were obtained as in the photographic dark room, though the difference was noted in that the animals in this experiment, gathered on the pan underneath the rocks, whereas in the dark room this preference was not shown.

According to Pearl ('02), the ventral surface of Planaria maculata is strongly positively thigmotactic; but this does not explain the tendency of the animals to rest in angles. This resting in angles he calls goniotaxis. The physiological condition of reduced tonus helps to determine whether or not the animal will rest on a smooth surface.

The increased resistance to movement may be one cause for stopping on the uneven surface tho the most important factor is probably light. Nevertheless, instances are known where the animal may stop in a bright light, tho such instances are rare.

From table 1 it is seen that rock four has an average of 34.59 animals per unit area. The surface of this rock in degree of roughness, is midway between the rough granular surface of rock number one (average 25.53) and paraffin-glass (average 30.18). Table 2 shows that the paraffin-glass surface (average 29.17) is the highest per unit area, followed by rock six (average 23.06) whose surface is of smooth weathered limestone. In tables 1 and 2, it will be noticed that surface number seven is of glass. This surface is horizontal, not vertical as is that averaged with the paraffin. If the average of this surface (16.25 in table 1 and 17.66 in table 2) were added to the surface average of the paraffin-glass (average 30.18 in table 1 and 29.17 in table 2), the total average of these surfaces would be 46.43 in table 1 and 46.83 in table 2. This is much higher than any of the rough surfaces. From this the conclusion may be drawn that the animals prefer a smooth surface.

Pearl ('02), in describing the method of locomotion of Planaria maculata says that the ventral surface of the body constantly secretes mucus in greater and lesser quanities. This is very sticky and under normal conditions adheres to the surface on which the animal reposes. Thus between the animal and the surface on which it moves there will be a constant layer of mucus. The beating of cilia in this mucus pushes the animal forward. Considering this explanation, then, more mucus may be secreted by an animal when passing over a rough surface than when passing over one that is smooth. Likewise a regular granular surface

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might not have quite so much effect as a very angular surface. Pearl ('02), also suggests that the reduced physiological tonus of the animal might cause it to rest on the rough surface.

To find whether or not more mucus was extruded and secreted when passing over a rough surface than over a smooth one, the following experiment was tried. A piece of ordinary window glass was scratched or grooved by a diamond glass cutter. The glass with the grooved surface was then placed in a large petri dish and covered with water. Some of the animals were then placed in the dish and allowed to move over the grooved surface. After an hour the animals were removed and the glass "developed" by immersing in Delafields' hematoxylin, which stained the slime blue. This gave better results than the method used by Pearl ('02), who used a solution of carmine. This was tried and the carmine particles by adhering to the mucus, showed the tracks but the mucus was not stained and hence a close study of it could not be made. The glass was then broken so that cross sections of the grooves could be examined with the microscope. A number of sections were thus examined but at no place could a greater slime secretion be noticed where the slime tracks crossed a groove.

Thinking perhaps that the width and depth of the grooves in a surface might cause some difference in the amount of slime secreted, the bottom of a large petri dish was covered by a layer of paraffin to an average depth of 5 mm. In this paraffin were cut grooves varying from 0.20 to 3 mm. in width and the same in depth. The dish was then filled with water and in it were placed some animals. The smaller grooves did not seem to reduce the rate of locomotion of the animals. However, when an animal approached one of the wider grooves, it would hesitate, raise up the anterior end of the body and move it to and fro, then pass across without touching the bottom of the groove.

After the animals had been undisturbed for two hours, they were removed and the water replaced by a solution of Delafields' hematoxylin. On examination of the stained slime tracks it was found that in few cases was there a greater amount of slime secreted. The slime strands did not descend to the bottom of the grooves but in most cases were continued on across as a sort of bridge. In some cases these strands were broken but even then no abnormal amount of slime was found. At the edges of the grooves, however, there were places where there seemed to be a greater amount of slime. These were the places, probably, where the animals hesitated.

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From these results it would appear that there is the same amount of slime on all surfaces and that no abnormal secretion is caused by a rough surface. Thus a change in the physiological condition of the animal as caused by the difference in mucus secretion, would not effect the thigmotactic reaction to surfaces of varying roughness and smoothness.

Pearl ('02), suggests that perhaps the rough surface offers more resistance to movement than does a smooth surface. This naturally would cause a slower rate of locomotion when an animal was passing over a rough than when passing over a smooth surface but since an equal amount of slime is secreted on all surfaces, there should be approximately the same rate for all surfaces. Accordingly the rate of locomotion was found for two surfaces, the one of rough sandstone, somewhat coarser than rock one, and the other glass.

A piece of white paper was marked off in 1 cm. squares and placed underneath a large petri dish filled with water. The surface of the rock was marked off in 1 cm. squares with waterproof India ink and around the edges were placed sides of pasteboard to keep the animals from wandering off the surface. The rock was placed in a galvanized pan which was filled with water to such a height that the surface of the rock was covered to a depth of 2 cm. The two surfaces were placed underneath the skylight in a room from which all other sources of light were cut off.

The four animals used in these experiments were kept in separate dishes carefully marked. When the animals were transferred from these dishes to the surfaces, a camel's hair brush was used in order not to injure them. In the course of these experiments, animal number one died, so that most of the tables show the results of the reactions of numbers two, three and four, all of which were active, healthy, individuals. The animals were placed on the surface singly, and a number of observations taken by means of a stop watch, as to the length of time required to move over 1 cm. of surface. No allowance was made for an approximate deviation of 2 mm. from a straight line in passing over 1 cm. of surface.

The data recorded in table 5 was procured thus. A number of observations of the rate of locomotion of each animal was taken on two consecutive days for the glass surface and on the two following days for the rock.

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TABLE 5

Animal	Rock 1st day		Rock 2nd day		Av. rate in mm. per sec.	Glass 1st day		Glass 2nd day		Av. rate in mm. per sec.
	no. of obs.	av. rate in mm.	no. of obs.	av. rate in mm.		no. of obs.	av. rate in mm.	no. of obs.	av. rate in mm.	
1	15	0.92	15	1.23	1.07	10	0.74			0.74
2	15	0.80	15	1.10	0.95	16	1.43	18	1.2	1.31
3	15	1.05	15	1.17	1.11	17	1.47	18	1.46	1.46
4	15	0.97	15	1.24	1.10	18	1.38	18	1.47	1.42

Observations showing the rate of locomotion. Observations taken on one surface only each day

Table 5 shows that the average rates per second of animals number three and four are the highest, being 1.11 mm. and 1.1 mm. on the rock and 1.46 mm. and 1.42 mm. on glass. This shows a difference in rate of speed per second between the glass and the rock of 0.35 mm. and 0.32 mm. Animal number two shows a difference per second of .36 mm. In these three instances the animals moved more rapidly on the glass. However, a further examination shows that an animal in twenty-four hours time will vary in rate of locomotion on the rough surface .31 mm. and on the smooth .21 mm. per second. Walter ('02), who tested the rate of speed on glass only, found that the rate varied from 1.22 mm. to .96 per second. So the variation in rate between the two surfaces can scarcely be attributed wholly if at all, to the difference between the two surfaces but to some other factor. This is further proven by a study of tables 6 and 7.

The data in tables 6 and 7 differs from that in table 5 somewhat. A number of observations on the rate of locomotion of an animal was taken first on one surface and immediately on the other. Several hours later another set of observations was taken. This lessened the chance for so great a change physiologically as to effect the speed. The results are seen in tables 6 and 7.

Table 6 shows at 8:00 the maximum difference of speed of all the animals, between the two surfaces to be .16 mm. per second. At 2:00 the maximum difference is .1 mm. per second.

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TABLE 6

Animal	time	Rock		Glass	
		no. of obs.	av. rate in mm. per sec.	no. of obs.	av. rate in mm. per sec.
2	8:30	8	1.15	7	1.21
3	until	7	0.98	7	0.92
4	11:00	9	1.17	7	1.01
2	2:00	7	0.99	9	0.99
3	until	7	1.02	9	1.12
4	4:00	7	1.01	5	1.11

Observations showing rate of locomotion. All the observations were taken on one day.

TABLE 7

Animal	time	Rock		Glass	
		no of obs.	av. rate in mm. per sec.	no. of obs.	av. rate in mm. per sec.
2	9:00	5	1.06	7	1.36
3	until	7	1.01	8	0.98
4	11:00	7	1.19	8	1.24
2	1:30	5	1.03	7	1.12
3	until	7	1.01	7	1.17
4	3:00	7	1.19	4	1.33

Observation showing rate of locomotion. All the observations were taken on one day.

Table 7, a day later, shows the maximum difference in rate at nine o'clock to be .3 mm. per second and at 1:30 o'clock to be .16 mm. per second. Further, in some cases the rate of speed is greater on the rock than on the glass.

The difference, then, in rate of locomotion between a smooth surface and a rough surface is so small as to be almost negligible. This further

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shows that the amount of slime extruded and secreted is not increased for a rough surface since this would probably reduce the speed of the animal.

According to Pearl, ('02), the method of locomotion in Planaria maculata is by means of the cilia beating in the mucus strands. Thinking that perhaps a study of the cilia and hypodermis might throw some light upon the thigmotaxis and locomotion of these animals, some work was done upon the histological structure of Phagocata gracilis.

Planarian tissue is one of the most difficult of animal tissues to study because it is so hard to fix properly and to stain. A fixative may give good results in one case and not in another. Five different fixing fluids were tried, hot Gilson's, hot and cold corrosive-acetic, twenty-five per cent solution of nitric acid, hot corrosive sublimate and a fixative recommended by Woodworth, ('91), made up of a saturated solution of corrosive sublimate in fifty percent nitric acid; of these, corrosive sublimate gave the best results. Of the various stains, borax-carmine, picrocarmine, neutral red, picric acid and carmine (aqueous), Delafields hematoxylin, Ehrlich's hematoxylin and eosin gave the best results. The sections were cut 6.6 microns thick. In all about thirty animals were studied.

The study was made principally of the hypodermis. On the ventral side, figure 2, it is made up of strongly ciliated columnar epithelial cells These contain large subcircular nuclei, nu, with evenly distributed chromatin granules. Definite cell walls between the cells could not be distinguished. Between the cells are spindle shaped, homogeneous bodies, the rhabditi, rb, which (Woodworth, '91) are developed in subcutaneous flask-shaped cells which are ectodermic in origin. These were supposed to be homologous to the nematocysts of the Coelenterata but later were supposed to be gland secretions. Underneath the hypodermis is a homogeneous layer, the basement membrane, bm. Under this is found the different muscle layers and the body parenchyma, figure 1.

The dorsal hypodermis, figure 3, is made up of columnar epithelial cells somewhat longer than those on the ventral surface. No cilia were found on these cells except near the edges of the animal. The rhabditi, rb, were much larger and more numerous than on the ventral hypodermis. They were found in groups of two or three. So thick were these that the nuclei of the cells, in many cases, were pressed out of shape and often concealed. The nuceli, nu, of the cells, when seen, appeared to have

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the same structure as those in the ventral side. Beneath the cell layer is found the basement membrane, bm. It is somewhat wider than on the ventral surface but has the same appearance. Beneath this membrane are the muscle layers and the parenchyma, mus. par. Imbedded in the parenchyma are numerous rhabditi mother cells, rb. mc, figure 1.

According to Woodworth, ('91) these rhabditi rapidly disintergrate when extruded into the water to form a slime which, by entangling the prey, aids the animal in procuring food. They may also be used for protection. Whether or not these rhabditi play any part in the locomotion of the animal, I was unable to determine.

In reviewing the literature on planaria there seems to be a difference of opinion among investigators regarding the distribution of cilia on the surface of the animal. Pearl ('02) finds none on the dorsal surface of Planaria maculata. Metschinikow ('66) and Kennel, ('79), found cilia covering the whole surface of Rhynchodesmus and Geodesmus but Zacharias, ('88), states that the dorsal surface of a variety of Goedesmus is bare. Vejdowsky, ('90), maintains the same for Microplana, the cilia in the latter cases being confined to the ventral surface or sole. Woodworth, ('91), found the cilia on Phagocata gracilis (collected near Cambridge, Mass.) to cover the entire surface of the body. I was able, tho I used the same technique as Woodworth, to find cilia only on the ventral surface and sides of the animal. However, these animals were collected in Falling Run, Morgantown, W. Va. This difference in localities may account for the variation in ciliation. Mosely, '74, explains that this absence of cilia on the dorsal surface of Bipalium may be due to the fact that the cilia on the dorsal surface of land planarians are weaker thru comparative lack of function and are more easily destroyed by reagents. However this is still an undecided question.

Summary

The results in tables 1 and 2 show that there is a preference shown for a smooth surface rather than a rough one.

Tables 3 and 4, are interesting in that they substantiate the results found by Olmstead, ('17), that unfed Planaria maculata are positively geotropic, that is to say, have a tendency to keep the ventral side toward the stimulus of gravity. Likewise these results show that *Phagocata gracilis* is strongly negatively phototropic.

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Since no mucus was found filling the grooves and cracks in the glass, it is clear that there must be no abnormal secretion of mucus and this is further strengthened by the results found in tables 5, 6 and 7, that there is an almost constant rate of locomotion on both a smooth and a rough surface. The last three tables also show that there is a variation in rate of locomotion which may be due to the different physiological states of the animal.

A histological study of the hypodermis discloses the fact that no cilia could be found on the dorsal side of the animal, which may be due to a difference in the variety of the variety of the animals, since Woodworth, ('91), found cilia on the dorsal hypodermis of the same species, collected in New England.

Conclusions

1. Phagocata gracilis is positively thigmotactic to a smooth surface.
2. This is not due to varying amounts of mucus secreted.
3. Phagocata gracilis is positively geotropic and strongly negatively phototropic.
4. The rate of locomotion is the same for both a rough and am sooth surface.
5. No cilia are found on the dorsal surface of Phagocata gracilis collected at Morgantown, W. Va.

University of W. Va.
Morgantown, W. Va.

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Explanation of Plate

Plate XI

Fig. 1. Transverse section of the animal anterior to the mouth, × 22.
Fig. 2. Portion of the ventral hypodermis, × 2000, approximately.
Fig. 3 Portion of dorsal hypodermis, × 2000, approximately.

Lettering

bm. basement membrane
cil. cilia
c. mus. circular muscle
hyp. hypodermis
l. cil. limit of cilia
l. mus. longitudinal muscle
mus. muscle
mus. par. muscle and parenchyma
nu. nucleus
par. parenchyma
ph. pharynx
rb. rhabite
rb. mc. rhabdite mother-cell

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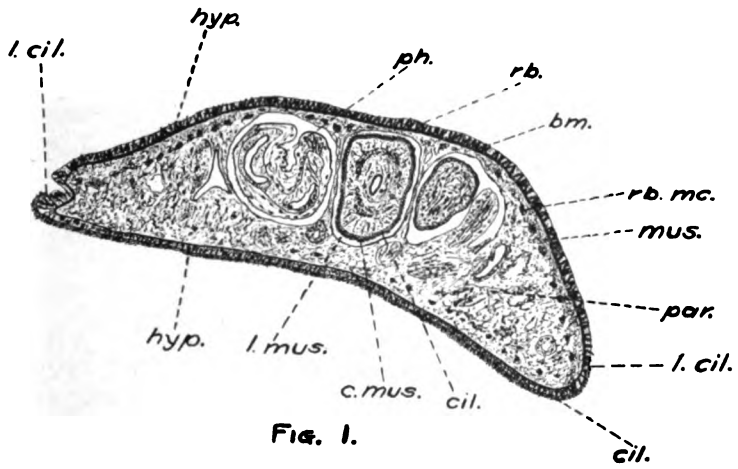

Fig. 1.

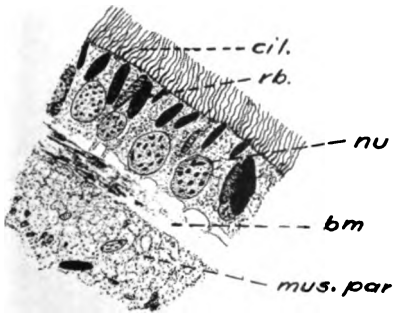

Fig. 2.

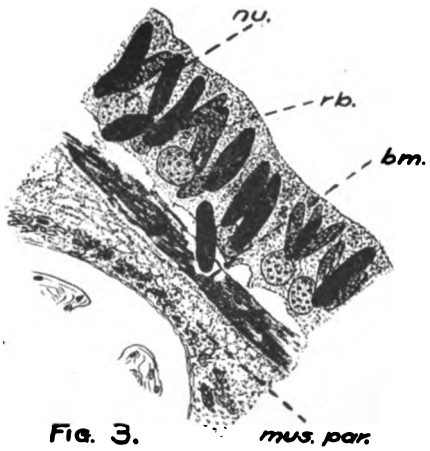

Fig. 3.

PLATE XI

WEIMER

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ADDITIONS TO OUR KNOWLEDGE OF *UNIONICOLA ACULEATA* (KOENIKE)*

BY

ERNEST CARROLL FAUST

The material of *Unionicola aculeata* (Koenike) on which this paper is based was secured from one of many specimens of *Lampsilis luteola* Lamarck, from North Judson, Indiana. The host was determined by Mr. Frank C. Baker, Custodian of the Natural History Museum of the University of Illinois. The general organization of the mite conforms to the type species described by Koenike (1890), but certain interesting variations and certain structures yet inadequately described deserve to be made a matter of record.

U. aculeata was originally described from Germany by Koenike, as similar in many respects to *U. crassipes* (Müll.) and in others to *U. figuralis* (Koch). It differs from the former in the exact structure of the palpus, in the number and distribution of the genital acetabula, and in the structure of the chitinous ovipositor. Wolcott (1899) describes this species from Michigan. The mite recorded by Soar (1899) as *Atax taverneri* has also proved to be *U. aculeata*. Piersig (1901) has made two subspecies of *U. aculeata*, separating the American form, *U. aculeata sayi*, from the European form, *U. aculeata aculeata*, on the basis of the number of the tarsal claws, comparative lengths of the leg segments, exact relations of the parts of the genital field, and a slight size difference.

The specimens collected by the writer conform to those described by Wolcott in most respects. In size they belong to Piersig's smaller group, since the female measures about 680μ long and the male measures about 640 μ long.

Wolcott's specimens have a terminal segment to the female palpus which is somewhat attenuate distad, ending in four small claws. In the writer's material the palpus has a more distinctly thickened terminal joint, with two finger-like claws and two thumb-like claws in the female and with two finger-like claws and only one thumb-like claw in the male. The penultimate joint of the palpus in Wolcott's material has a needle spine which is not present in the North Judson material. In the female of this material the basal segment is in each case the thickest of the six.

*Contributions from the Zoological Laboratory of the University of Illinois under the direction of Henry B. Ward. No. 111.

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The joints are progressively narrower and longer from base to tip. In the female there are three conspicuous spurs on the ventral side of the basal joint, and two undivided sickle-shaped claws at the end of each tarsus. Long needle spines are prominent on segments two to four. Soar (1899) figures the claws of the first leg as cleft, a feature which Wolcott (1899) describes for both *U. aculeata* and *U. crassipes*, while those of the writer's material are entire as in legs two to four.

Perhaps the most interesting feature of the entire body structure of *U. aculeata* is the heteromorphic fourth leg of the male. On this appendage both the joints and spines are curiously modified so that they present a striking ornate appearance (fig. 5). The tarsus is a long attenuate flat plate as in the female, with two undivided terminal claws and two accessory spines. The main shaft of the tarsus is free from spines. The tibia is short and thick with seven needle spines, two large heavy spines and one small spine. The third segment has three large and three small blunt spines, in two parallel lines at the outer edge of the joint. The fourth and fifth joints are both shorter than joint three altho not as short as the tibia. They are both supplied with several short bristles. The basal joint is irregularly sculptured and bears four spines.

The genital field of the female resembles closely that described by Wolcott, altho the cleft between the two plates is not distinguishable as a separate structure and no posterior papillae are found in the writer's material. The external male genital organs (fig. 3) are very complicated, consisting of an ornate sculpture of chitin, to which prominent muscle bands are attached.

A thoro study of the new material and comparison with that described by Koenike, Wolcott, and Soar, shows the wide range of variability within the species, while at the same time it discredits Piersig's separation of the species into two subspecies. It seems much more desirable to regard the species simply as highly variable rather than to maintain a subdivision, since the lesser similarities and differences grade into each other almost imperceptibly in various specimens.

Unionicola aculeata has been credited by some as a parasite of the Unionidae and by others as free-swimming. Koenike (1915) has shown that it is free-swimming during a considerable part of its life and seeks the mussel at times of metamorphosis and propagation. This fixes our knowledge of the periodicity of the parasitism, but leaves us in the dark with regard to the degree of parasitism. The mites described in this

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paper were found embedded in the subdermal connective tissue of the mantle and foot of the mussel (fig. 6). Their position was related to no definite axis of the host. Around it was a thin tissue cyst. In no case were they found to have injured the host outside of the cyst. Thus, altho an endoparasite, the evidence shows it to be only a temporary lodger.

University of Illinois.

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Explanation of Plate XII

Fig. 1. Dorsal view of female palpus, X 226; fig. 2, ventral view of first leg of female, × 140; fig. 3, male genital organs, X 140; fig. 4, female, ventral view, × 100; fig. 5, heteromorphic third leg of male, X 140; fig. 6, section thru outer portion of foot of *Lampsilis luteola*, with encysted *Unionicola aculeata*, × 140.

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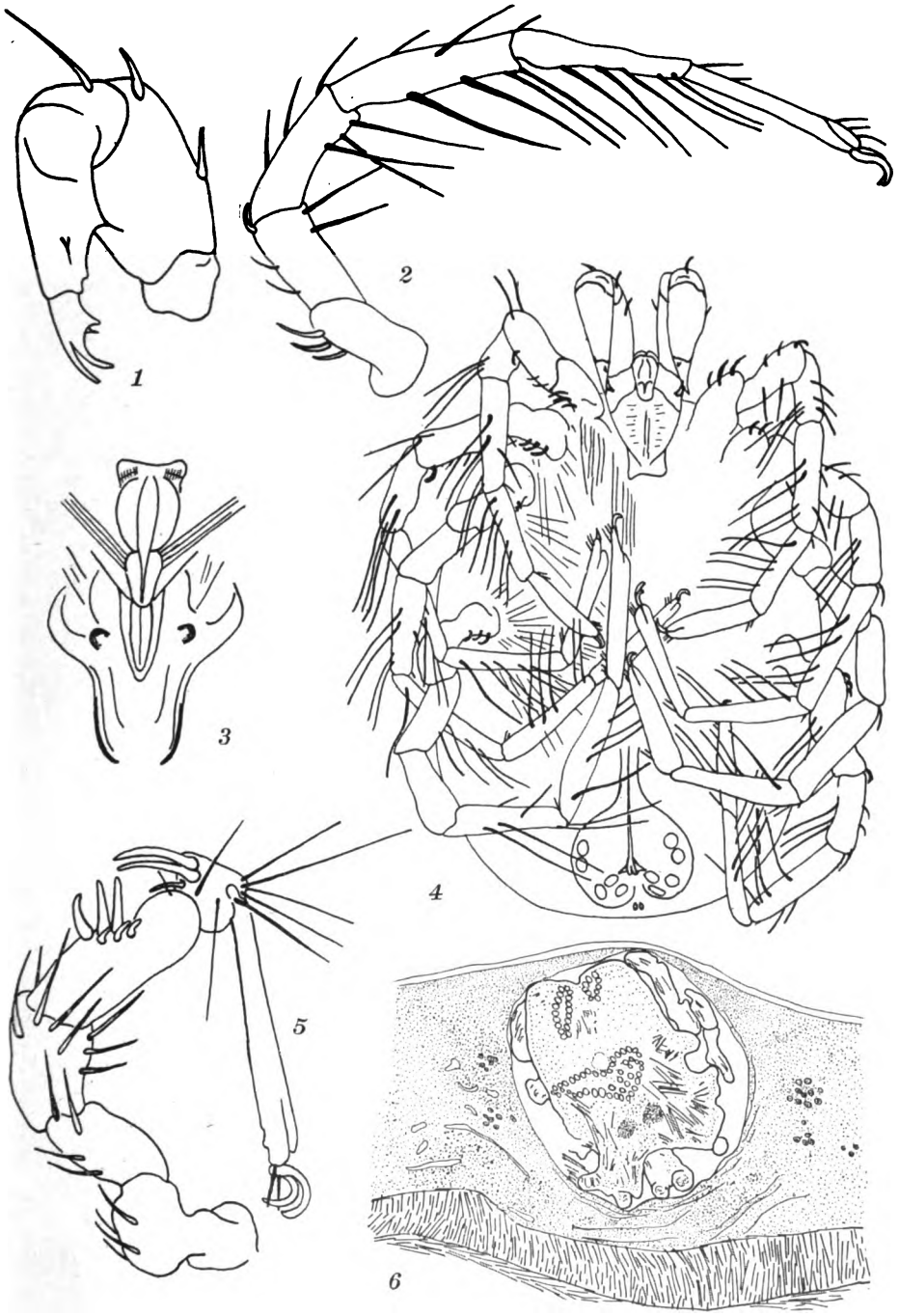

PLATE XII

FAUST

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DEPARTMENT OF NOTES AND REVIEWS

It is the purpose, in this department, to present from time to time brief original notes, both of methods of work and of results, by members of the Society. All members are invited to submit such items. In addition to these there will be given a few brief abstracts of recent work of more general interest to students and teachers. There will be no attempt to make these abstracts exhaustive. They will illustrate progress without attempting to define it, and will thus give to the teacher current illustrations, and to the isolated student suggestions of suitable fields of investigation.—[Editor.]

METHODS FOR STUDYING LIVING TREMATODES

Studies on the living animal are of great importance in morphological work on the Trematoda. Such studies have been entirely neglected by most workers on the group. I believe that an increased use of living material in the study of trematodes will make it possible to advance the knowledge of the group more rapidly and to avoid many errors. Material of living larvae and adult trematodes is easily obtained for class use, and its use adds greatly to the students' interest. Abundant material of sporocysts, rediae and cercariae can usually be obtained by the examination of freshwater snails and material for the study of adults from the intestines or lungs of frogs or snakes.

In miracidia or cercariae careful studies on locomotion reveal interesting specific characters, which may give hints of the type of host in which further development is carried on. The power of extention and contraction is so great in most trematodes, especially in larval stages, that a true conception of size and shape can be only gained from the living animal. The pattern of the excretory system can only be made out from living material. In fact it is almost impossible without careful studies in the living condition to define sufficiently the structure of a cercaria to insure specific determination. The amount of detail of structure which can be quickly obtained from the study of living trematodes is often surprising. In one small distome about 2 mm. in length it was possible from one living specimen not only to work out the connections of the reproductive ducts and to gain some idea, from the direction of the beat of the cilia, of the functioning of the parts, but also to make a camera lucida drawing under the oil immersion of the connections of the female ducts.

Agamodistomes and adults for live study should be transferred to slides in normal saline solution and covered with thin cover glasses.

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The water should be slowly removed from the preparation with a piece of blotting paper until the pressure of the cover glass slightly flattens the fluke without injuring any of its structures. Such a preparation, when carefully sealed with vaseline, will often remain a whole day in perfect condition for study and can be examined even with an oil immersion lens. Sporocysts, rediae and cercariae are usually found in large numbers in the digestive gland of the gasteropod host. The fully developed cercariae should be mounted for study in the water from which the snails are obtained, and the sporocysts, rediae and immature cercariae in normal saline. By slowly removing the water from beneath the cover glass with blotting paper, cercariae so small that they are almost invisible to the naked eye can be slowed down and flattened so that they can be studied under the highest powers of the microscope. When dealing with small forms it is easier to make new mounts as the one being studied becomes too dry, than it is to try to make a preparation more permanent.

At every stage of compression different structures are brought out. Just before a cercaria goes to pieces in the process of drying the smaller tubules of the excretory system are distended and the movements of the flame-cells accentuated, so that they become clearly visible. By careful observations and the use of a number of preparations the number and position of the flame-cells and the relation of the tubules can be gradually traced until the whole pattern of the excretory system is understood and recorded. By this method I have been able to work out the excretory systems of miracidia, rediae, agamodistomes and small adult trematodes. The pattern of one system containing one hundred and twenty flame-cells was successfully solved. I know of no other way by which such a complicated excretory system could be worked out. The compound binocular microscopes recently put on the market have proven very helpful in the type of studies described above. This instrument is easy on the eyes and gives depth to the object observed. Several intra vitam stains have been tried. So far no method of intra vitam staining has been found which gives a better picture than the unstained animal.

WILLIAM WALTER CORT.

University of California.

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A SUBSTITUTE FOR EUPARAL

In his original paper (La Cellule, 23, 427, 1906) Professor Gilson omitted the method of preparing this medium because of its difficulties and referred the matter to Grübler and Holborn. Under present circumstances it will perhaps not be a breach of etiquette to submit a method which yields a medium with similar index and properties. According to Gilson, Euparal is a solution of Sandarac in a mixture of Eucalyptol, Paraldehyde, and Camsal.

Paraldehyde and eucalyptol can be purchased of any chemist's supply house. They should be dry and neutral. If purchased from the druggist, who will probably substitute oil of eucalyptus, they should be redistilled, reserving the fraction, 119° to 124° for paraldehyde, and 175-177° for eucalyptol. The druggist's stock is in both cases about one-third something else.

Camsal is made by taking three parts of salol and two parts of camphor, and warming gently until completely liquid. Thereafter the mixture remains liquid, but should be kept well stoppered.

Sandarac as purchased is full of dust and ants. It may be purified as follows: 30 grams of sandarac are placed in a 200 cc. flask and 150 cc. of absolute alcohol added. Let stand with occasional shaking until dissolved. This mixture is rather sensitive to water vapor from the air and should be handled accordingly. Filter the solution thru a good filter paper into a 300 cc. flask. This is best done by resting the short funnel in the neck of the receiving flask and covering the whole with a bell jar under which some anhydrous calcium chloride is placed. Filtration is much more rapid than Mayer's albumin. The receiving flask is now fitted with a two-hole stopper. Thru one hole passes a glass tube in which a cotton filter has been placed; to catch dust, the rubber connections must of course be clean. This filter is connected to a calcium chloride drying tower to remove water vapor. The other hole is connected thru a receiving flask, if the alcohol is to be recovered, and thence to an aspirator. Air is passed while the solution of gum is warmed gently to 50-60°. Do not bubble air thru the solution, that being unnecessary and injurious. As the solution becomes thicker the temperature may be slowly raised to 70° and finally to 80° to remove the last of the alcohol. When the gum is moderately brittle on cooling the operation is ended.

To the gum in the same flask, add 20 cc. Eucalyptol, 10 cc. Paraldehyde, 10 cc. Camsal, cork and warm gently until a homogeneous solu-

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tion is obtained. This gives a medium with an index n=1.483 to 1.486. The index can be raised or lowered slightly by varying the proportions used in making the solvent. Thus, the indices of the ingredients are about: Eucalyptol 1.456; Paraldehyde 1.39, this varies with the preparation used; Camsal 1.534; Sandarac 1.525. The essence d'euparal is, of course, the solvent mixture used above. The green tint mentioned by Gilson as due to a certain copper salt is probably copper abietinate which can be had of Merck or can be made of sufficient purity by any student in the organic chemistry laboratory.

In my experience there is less difficulty in preparing this medium than with some of the staining mixtures. It takes time but also little attention. Slides mounted several months ago are in excellent condition and as near as one can judge the medium acts like Euparal. Sections can be mounted from 80% alcohol, either with or without passing thru the essence.

E. S. SHEPHERD.

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Washington, D. C.

CHROMOSOMES OF RANATRA SP?

During the summer of 1914 while working on the male germ cells of another type, I prepared and sectioned some testes from a species of *Ranatra* collected about Madison, Wis. The large number of chromosomes together with what seemed to be a very puzzling polymorphism of spermatocytes induced me to defer a further investigation till a later time. Recently the work upon this form has been resumed and has progressed to a point where a preliminary and tentative statement may be made.

The testes in the later nymph stages are especially valuable for sectioning as they present in many cases the whole history of the germ cells from the last spermatogonial divisions to mature spermatozoa. Sex organs from adults collected in the spring, and up to mid-summer are also generally favorable, but specimens taken in late summer and fall show very few division stages.

My first material was composed of several testes from animals collected in mid-summer and at that time believed to belong to but one species. All of these were prepared together for study. Observations

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upon this mixed material show that there are at least two types of testes as regards the chromosomes.

The first type has spermatogonia with 40 chromosomes of various sizes; primary spermatocytes with 21, all of which divide equally; secondary spermatocytes with 21, two of which do not divide but pass directly into different spermatids each of which then possess 20 chromosomes. The two chromosomes which do not divide in the second division act as a typical XY pair and always appear near the center of the chromosome group in this division but their behavior is not sufficiently different to enable them to be identified before this stage.

The second type has spermatogonia provided apparently with 8 or 10 more chromosomes than the first type. The primary and secondary spermatocytes seem to have as a distinguishing mark a group of very small chromosomes near the center of the larger group. Neither the number nor the behavior of the chromosomes in the spermatids has been determined although some interesting conditions are suggested by the rather meager observations made to date.

Another interesting though not necessarily important fact is that among the individuals collected in July few possessed testes of the second type while a large percentage of those collected in September did possess cells of that type.

In addition to the interest attached to spermatogenesis these forms seem to offer an opportunity to determine possible correlation between the chromosome differences and somatic variations as soon as the individual origin of the two kinds of germ cells can be determined.

A. M. CHICKERING.

Beloit College.

NOTES ON COLLECTING AND MOUNTING ROTIFERS

C. F. Rousselet, the veteran English naturalist (J. Q. M. C., Nov. 1917) sums up methods which he has worked out for collecting, handling, preserving and mounting rotifers.

For a collecting stick he recommends a walking stick with a telescopic joint, with a ring net 6x5½ inches and 6 inches long, made of bolting silk No. 15 or 16. Silk lasts longer than mull and does not clog or shrink as it does.

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The bottled materials collected are placed, on reaching home, in aquaria with 7x7 inch parallel sides one and one-fourth inch in depth from back to front. After a few hours the debris will have settled, and if a strong light be placed at one face of the aquarium the free-swimming rotifers will collect on the side toward the light, and can be discovered with a lens and be picked up with a pipette. In solid watch glasses these general collections can be examined quickly for new species with the low power of the binocular and unfamiliar forms transferred to the live-box or the micro-glass trough for special study.

Narcotising the mass of rotifers in the watch glass is readily effected by 1% cocaine. They may be killed and fixed by a drop of ½ to ⅛% osmic acid. They should be exposed to the osmic acid for a minute only and then removed to formalin of 2½% strength, changing it several times until well washed.

The sorting out of different species is done under the binocular by means of a bristle mounted in a suitable handle. They are then picked up with a fine pipette and placed in an appropriate micro-cell, and finally mounted in 2½% formalin.

The ringing of the micro-cells may be done as follows: first a thin ring of picture copal varnish; then several coats of Heath's cement (gold size-shellac-India rubber); finally finishing with three more coats of gold size.

METHODS OF PRESERVING CERTAIN MARINE BIOLOGICAL SPECIMENS

F. Martin Duncan (J. R. M. S., Dec. 1917) brings together methods which he has found most practical and successful in preserving marine plant and animal life and in preparing it for microscopic examination. Many of these methods are standard; but summarizing some of them may be of value.

Anaesthetising

Place the smaller and specially sensitive medusæ in just sufficient sea-water for free expansion and swimming, and add two drops of 1% solution of hydrochloride of cocaine, gently stirring with a glass rod. Repeat at five minute intervals until the tentacles do not contract when gently touched. Add 10-20 cc. of 4% formaldehyde solution, stirring for several minutes. Store in 10% formaldehyde. Do not allow specimens to remain in cocaine longer than absolutely necessary before adding the formaldehyde, as the former softens the jelly of the medusæ.

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The author regards cocaine as, on the whole, the best anaesthetic for the most of the smaller forms of marine life. Solutions of coacine must be made anew since they do not keep well—becoming filled with fungoid growths.

Hydroid zoophytes, simple and compound ascidians, holothurians, anemones, and the like may be stupefied effectively with menthol. This is slow in action and does not simulate to contraction. The animals are submerged in clean sea-water and methol crystals are strewn over the surface. Their solution is slow, and in twelve or twenty-four hours depending on the size and sensitiveness of the animals and the amount of water, the specimens will be narcotised in an extended position, and may then be killed and fixed in any suitable fluid.

Fixing

The author prizes Bouin's fluid (Picric acid, saturated aqueous solution, 75 parts; formalin 25 parts; glacial acetic acid, 5 parts) as the best fixative for histological purposes. It has great power of penetration, kills quickly, and fixes well. It allows after treatment of the most varied sort. Next in desirability he considers saturated solution of corrosive sublimate.

Weak osmic acid (a few drops of a ½% solution added to the water in which the organisms are) is suggested for marine Protozoa. Radiolaria are effectively killed and fixed in corrosive sublimate. Sphærozoa give good results in equal parts of sea water and 70% alcohol with a trace of tincture of iodine added.

For Echinoderm larvæ an exposure of four minutes to a cold saturated solution of corrosive sublimate is recommended. For whole mounts dilute cochineal stain—as Mayer's alcoholic cochineal formula.

Small sponges are placed, on collection, in 1% solution of osmic acid and left there for five minutes, then transferred to strong alcohol and changed twice. Stain sections in Mayer's alcoholic cochineal.

Compound ascidians with contractile zooids may be handled to advantage by placing in clean sea water, narcotizing with methol, and then plunging for three to ten minutes in glacial acetic acid. Wash in 50% alcohol and pass thru successive grades to a preserving strength. Use no metal in the operation.

For small crustacea, both larvæ and adults, first treatment with 5% formaldehyde in sea water is recommended. Transfer to 70% alcohol.

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For demonstration mounts it is necessary to guard against overstaining. Weak alcoholic picro-carmine cleared in turpineol is advocated as a means of staining.

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JULY
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T. W. GALLOWAY,
Secretary-Editor.

Beloit, Wisconsin.

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TRANSACTIONS
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Vol. XXXVII JULY, 1918 No. 3

OBSERVATIONS ON REPRODUCTION IN CERTAIN PARTHENOGENETIC AND BISEXUAL NEMATODES REARED IN ARTIFICIAL MEDIA*

By Paul S. Welch and L. P. Wehrle

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*Contribution from the Entomological Laboratory, Kansas State Agricultural College, No. 33.

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INTRODUCTION

Nematodes present material for a wide variety of investigations. Not only is the group very incompletely known, especially the free-living species, but much also remains to be learned about their life processes. One of the fertile, outstanding possibilities for investigation relates to the remarkable features of reproduction presented by these animals. Maupas ('00) and others have pointed out the striking diversity in the methods of reproduction and the possibilities of this group in extending the knowledge of sex determination, origin of parthenogenesis, origin of bisexuality, and the nature of hermaphroditism. The development of methods whereby these animals can be maintained in pure cultures for indefinite periods adds to their value as material for critical study. In this paper, the writers present results of observations on cultures maintained continuously for more than three years. The data herein presented are general in character and the results of study of certain special problems will appear at a later date.

The writers wish to express their indebtedness to Dr. N. A. Cobb for the identification of the specimens used in this work and to Mr. A. L. Ford who, during the absence of the writers, maintained the cultures through the summer months of 1917.

METHODS OF CULTURE

In connection with the work on which this paper is based, the writers had occasion to use a number of methods for rearing nematodes in artificial media. Previous investigators devised means whereby certain free-living and parasitic nematodes could be maintained for a number of generations under laboratory conditions and some of their results were used to advantage in the present work. In some cases, previously known methods have been modified, while in others, new procedures were developed. It is obvious that, in order to study these animals

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successfully, they must be reared under conditions other than those of the natural environment and the fact that they can be cultured in artificial media for indefinite periods of time makes it possible not only to observe continuously the details of activities and of life history but also to carry on a large variety of experiments. The writers maintained continuous cultures of *Diplogaster ærivora* and *Cephalobus dubius* for over three years. Cultures of the latter are still under observation and are all descendants of one original stock. Since the published methods of rearing the smaller nematodes appear in scattered sources, the more important ones will be given brief notice here. In addition, the methods employed by the writers will be discussed in detail.

Methods of Other Investigators

Conte ('00a, p. 374) cultured *Rhabditis monohystera* by employing as nutritive media "la colle de pâte trés epaisse les solutions de peptone et les tranches de pomme de terre. Ces cultures etaient faites dans des assiettes couvertes et, d'autre part sur porte-objets en isolant un femelle fécondée dont j'etudiais ensuite la descendance." He was also able ('00b, p. 376) to rear *Diplogaster longicauda* "dans la colle de pâte."

Metcalf ('03, pp. 93-98) reared a nematode of uncertain identity (*Rhabditis brevispina ?*), found in diseased corms, young stalks of *Crocus* and cuttings of *Petunia*, *Coleus*, and *Geranium* on plates of asparagus agar. Sterile agar was unsuitable, but a one per cent asparagus juice agar, inoculated with a *Fusarium* and the bacteria which occurred with the original stock of the nematodes and allowed to decay for about two weeks, was satisfactory after it had been heated, filtered, and sterilized. In this medium, sterilized eggs developed rapidly and normally. A certain fluidity of the medium was found to be desirable.

Potts ('10, pp. 443-446) readily cultured certain free-living nematodes (*Diplogaster maupasi*, *Rhabditis gurneyi*, *Rhabditis elegans*, *Rhabditis duthiersi*, *Rhabditis sechellensis*) in drops of nutrient media in watch-glasses closed with a vaselined glass cover. Solutions of "brown" and "white" peptone, used almost exclusively as the media, were allowed to putrify until a cloudy growth of bacteria had formed throughout them. In the presence of large numbers of bacteria, the nematodes thrived but, in sterile solutions, growth was suspended and eggs were deposited

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only at long intervals. Potts did not record the strength of the peptone solutions—a matter of importance since, as will be shown later, an excess of peptone has a deleterious effect on the worms. Two or three successive generations were reared in a saturated solution of gelatin in water, and mature individuals were secured from eggs in "solutions of amides like tyrosin and leucin" but restriction of both growth and egg production indicated that these media were inferior to peptone. Beef infusion was also used to a very limited extent.

Oliver ('12) cultured an unknown nematode, found in the exudate about the genitalia of dead guinea pigs, in a medium made by inoculating a little of this exudate onto moist earth and slants of Musgrave's amœba agar, kept at a temperature of about 75° F. Successful subcultures were also made by using plain agar and ascites agar, plain agar and amœba agar giving the best results.

Johnson ('13, pp. 612-618) was able to culture *Rhabditis pellio*, a parasite of the nephridia of the common earthworm (*Lumbricus terrestris*), by methods somewhat similar to those used by Potts, Conte, et al. Filtered tap water or salt solution in watch-glasses to which were added certain food materials constituted the basis of the cultures. The watch-glasses were either covered individually by means of a vaselined glass cover, or else several, without covers, were placed in a humid chamber which gave a larger air space but minimized evaporation. The culture medium was replenished from time to time. Experiments with Witte's peptone showed that a nearly saturated solution quickly proved fatal to the nematodes and the same result accompanied weaker solutions until a 0.15 per cent strength was reached. Although very dilute, this solution proved satisfactory for one series of cultures, but afterwards it was almost uniformly unsuccessful. A one per cent. hay infusion was successful for only one series. Meat extract, decaying meat, a solution of urea, and a hay infusion sterilized and inoculated with soil bacteria were all virtually useless. Decaying earthworm had an advantage over peptone in nutritive value, but presented the disadvantages of requiring special treatment to prevent introduction of nematodes already present in the worm; of becoming increasingly opaque as decay advanced, thus rendering examination difficult; and of occasionally becoming foul smelling and densely clouded, causing the death of all of the nematodes. The best medium was prepared by removing the alimentary tract from freshly killed *Lumbricus terrestris* and placing the body in a test tube

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which was then plugged with cotton-wool, heated in a steamer and kept at a boiling-point for about two hours, decanted, filtered, and inoculated with the bacteria which were associated with the worm in the soil. These bacteria were secured by allowing a fresh *Lumbricus terrestris* to decay in a small amount of water to which a little earth had been added. This medium also possessed the disadvantage of occasional development of bad odor and opaque scum, leading to the death of the entire culture.

Byars ('14) made use of a synthetic medium, Pfeffer's nutrient agar, in connection with some studies of the plant nematode *Heterodera radicicola* and found it suitable for the preliminary development of the worms up to their penetration into the tissues of experimental seedlings grown in the same medium. Most of the individuals which remained outside of the root of the seedling died within a few days, although a few remained active for more than a month. The latter, however, did not undergo their normal development into adults. It will be shown later that this medium is much more successful for certain other species.

Merrill and Ford ('16, pp. 121, 126) found that *Diplogaster labiata* flourished in water cultures to which had been added portions of the macerated bodies of the beetle *Saperda tridentata*—the host of this nematode. They state that "Different substances were tried with varying success, but macerated beetles placed in water seemed to be the most satisfactory" but these "different substances" were not specified. They also reared *Diplogaster œrivora* in similar preparations.

Methods of the Writers

Equipment

The writers had occasion to employ a variety of culture methods demanding a corresponding variety of equipment. Most of the cultures were maintained in cells constructed as follows: Cylindrical glass mounting-cells with ground edges were sealed to ordinary glass microscope slides by means of pure vaseline and covered with a circular cover-glass, the diameter of which was slightly greater than that of the glass mounting-cell. The cover-glass was also sealed on with vaseline. For stock cultures, the most suitable glass mounting-cell was found to be one having a diameter of 20 mm. and a height of 10-15 mm., preferably 15 mm. These tall cells made it possible to maintain a greater volume of the liquid culture medium, thus providing for a larger stock and, more im-

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portant still, minimizing the danger of cultures drying out since it was discovered that they could not be sealed tightly but demanded at least a small opening at the top. They offered the disadvantage of being too high for examination with anything but very low powers of the compound microscope. It was possible, however, to use the binocular microscope, and since there was little occasion to use the stock cultures for purposes other than maintenance, this disadvantage was of little consequence.

Cultures maintained for experimental or detailed observational work were kept in glass mounting-cells of another size, viz., diameter, 20 mm., height, 6 mm. Such a cell surrounded a space large enough to enclose a drop of the culture medium without involving the danger of the latter coming into contact with the vaselined edges of the cell, a precaution which had to be observed rigidly. That height also permitted examination with the ordinary low powers of the compound microscope without removing the cell from the supporting slide.

Many of the glass mounting-cells used in this work have walls 1 mm. thick. Cells having walls of 2 mm. thickness were found more advantageous, especially for stock cultures, owing to the greater vaselined area in contact with the slide, thus minimizing the danger of the watery medium leaking out and permitting the culture to dry up. The thinner cells were in more constant use owing to the difficulty of obtaining the thicker ones, and, if care was taken to thoroughly vaseline the ground edges and press them down onto the slide, they were quite satisfactory.

In special cases when greater space or capacity was desired, small stender dishes were used. A limited use was made of a group of glass mounting-cells sealed to the bottom of a Petri dish. For rapid observation of isolated individuals, Petri dishes alone were used for one series, large drops of the nutrient medium retaining their identity when placed on the dry, clean glass but not in contact with each other.

At times, culture slides of two kinds were used in place of the glass mounting-cells mentioned above. One form is the heavy plate glass, 76 x 26 mm. slide, 5 mm. thick and with a central, circular depression 4 mm. deep, and covered by sealing on a circular cover-glass with vaseline. This form of receptacle was satisfactory in many respects but was not usable for individual cultures because of its depth. Furthermore, it is more expensive than the glass mounting-cell. The other culture slide is the ordinary 76 x 26 mm. form, having a central, shallow

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concavity with maximum depth of about 1 mm., closed by a vaselined circular cover-glass. This type is useful for individual cultures.

Needles for transferring eggs, larvæ, and adults from one culture to another were in constant use. These instruments were made by setting into a small handle a fine needle, the point of which had been bent into a small recurved hook. It was found convenient to have several of these needles whose points were bent at different degrees. Ordinary insect pins of sizes 0 and 00 were used to some extent but the most useful form was the Japanned steel "Minuten Nadeln" commonly used for pinning minute insects. These pins made especially useful needles because of their very small diameter and extremely fine points, thus facilitating their manipulation under the high power of the binocular microscope and their use in transferring the minute nematodes.

Transferring brushes, which for part of the work were used instead of needles, were made from small camel's hair brushes by carefully cutting out the brush until a very small central tuft of 3-5 hairs remained. Such a brush was often used for transferring eggs.

Pipettes of the ordinary form were in common use for handling the various fluids employed in the work. In order to avoid contamination of the cultures, it was necessary to keep these pipettes properly labeled and thoroughly clean. A special form of pipette for removing specimens or for removing deteriorating culture fluids was made by drawing out in a flame the open end of an ordinary straight pipette until the opening was only about 0.3 mm. in diameter.

Media

In culturing nematodes, it is necessary to use some substance which will serve directly or indirectly as food for the animals. The writers have tried out a number of substances some of which have been distinctly successful. Since the same substances were not in every case employed for both of the species reared, the culture methods of each will be discussed separately.

Practically all of the culture media were more favorable under conditions of dilution, in fact, some of the most successful ones demanded extensive dilution, otherwise they were inimical to the worms. Distilled water was uniformly used, thus eliminating the danger of contaminating the cultures from that source, as would be the case with tap-water. Preliminary experiments with both *Cephalobus dubius* and *Diplogaster*

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ærivora showed that all stages of the life history lived for some time, several days in many cases, in distilled water alone, and when a small amount of nutritive substance was added, the medium became suitable for continuous culture. Apparently, the usual toxicity of ordinary distilled water had no deleterious effect on the worms.

Diplogaster ærivora.—Since the original stock of this species was found in the eggs of grasshoppers, the yolk of these eggs was at first used as a medium and, as might be expected, gave good results. Eggs from a number of species of grasshoppers appeared to be of equal value. Owing to the occasional difficulty or inconvenience in getting grasshopper eggs, the eggs of certain Coccinelidæ, of the Colorado potato beetle, of certain unidentified insects, and the ovaries of grasshoppers were successfully substituted. Furthermore, the softer tissues of young grasshopper nymphs, army worm pupæ, variegated cutworm pupæ, Hessian-fly pupæ, and pupæ of a number of other insects were found to constitute good media when prepared in the proper way. It is very probable that a wide variety of insect eggs and tissues would serve this purpose equally well.

The procedure in the use of the above mentioned substances depended upon the kind of culture desired. Most of the stock cultures, as well as the majority of the life history cultures, were maintained in the glass mounting-cells already described. When grasshopper eggs were used, they were first carefully cleaned externally and stripped of their shells, since fragments of the latter when present tend to render the examination of the culture more difficult. The contents were placed in a small amount of distilled water and thoroughly triturated in order (1) to distribute more uniformly the yolk throughout the culture and render it more readily available to the nematode, and (2) to facilitate the examination of the material for evidence of previous nematode infestation, thus aiding in the maintenance of pure cultures. This precaution is necessary since small nematodes often occur on the surface and apparently within some of these insect eggs and pupæ. The writers found them in connection with Hessian-fly puparia, both on the surface and apparently in the pupæ, although in the latter case there is the possibility that they might have been included by manipulation. However, Marchal has reported Osborn, '98, p. 41) nematodes in the puparia of this insect. Insect tissues mentioned in a foregoing paragraph were sometimes used in a

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similar way, removing, of course, all fragments of the integument. The proper amount of the diluted nutriment was placed in the glass mounting-cell which already contained the appropriate amount of distilled water.

The method of adding the food materials thus prepared was determined by the purpose of the culture. For stock cultures, two or three drops from a pipette were added every 3–5 days to the 1–1.2 c.c. of distilled water in the mounting-cell. A larger amount of food could be added without serious results to the organisms but it was not needed for nutritional purposes, and it had the disadvantage, at least in certain cases, of tending to increase the opacity of the culture.

When there was any occasion for using large quantities of the material for a medium, good results were secured by putting sterilized soil in a covered stender dish, moistening it liberally with distilled water, and transferring to it a pupa or other food object which was then perforated and inoculated with the nematodes from a stock culture. These cultures lasted for considerable periods of time, developing nematodes in quantities.

While the above-described methods were satisfactory, it was inconvenient in the maintenance of a long series covering different seasons of the year to keep a constant supply of the food materials at hand and to have a continuous stock of any one kind for experimental purposes. For that reason, a search was made for some food which is approximately constant in composition and easily available at any time. Among other things, the yolk of hen's egg was tried and found successful. Experiments with different amounts of this substance showed that, in a culture containing 1–1.3 c.c. of distilled water, the nematodes thrived when the quantity represented by three or four dippings of the points of ordinary forceps into the yolk was added. Almost any larger amount may be used but increased opacity and the occasional undue putrefaction make it undesirable. Amounts of food represented by one or two dippings of the forceps were, in general, found to be distinctly inferior in results and evidently represent too poor a culture. A single hen's egg supplied food material for a number of days. A small opening, approximately 1 cm. in diameter, was made through the shell and the adjacent albumen allowed to escape. Sterile forceps were used to lift out the desired quantities of the yolk and then the opening in the shell was sealed over with a gummed label or piece of gummed cloth and the egg kept in a cool

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place. When needed again, the gummed covering was removed, the desired quantity of yolk secured, and the opening again sealed. The amount of yolk adhering to the points of forceps is variable but the method was found to be both rapid and practical in the operation of ordinary cultures.

Liebig's extract of beef, diluted to different degrees with distilled water, was tried as a nutrient medium and found to be usable so far as maintaining the animals was concerned but its partial opacity impaired its usefulness.

Since Byars ('14, p. 323) found Pfeffer's synthetic agar useful in culturing certain nematodes parasitic on plants, it occurred to the writers to experiment with it as a possible medium for rearing *Diplogaster ærivora*. The medium used was as follows: calcium nitrate, 4 grams; potassium nitrate, 1 gram; magnesium sulphate, 1 gram; potassium dihydrogen phosphate, 1 gram; potassium chloride, 0.5 gram; ferric chloride, trace; distilled water, 6 liters; and agar, 12 grams. The formula used by Byars calls for powdered agar but the writers used the shredded agar cut up into small bits. This fluid was used directly from the stock solution without change and, while it appears from all the trials that it is somewhat inferior to certain other media used, nematodes were maintained in this solution for long periods of time. According to the experience of the writers, cultures in this medium, when properly cared for, are fairly satisfactory, but since there was some evidence of inferiority, parallel series of tests were carried on for 20 days, comparing the relative merits of Pfeffer's solution and hen's egg as media. Using the rate of development and reproduction as indices of the value of the media, four of the five series showed evidence of the superiority of the hen's egg, one of the five showing a slight advantage for the Pfeffer's solution.

Maceration cultures used by the writers have already been described and it seems very probable that, barring the danger of contamination, they might be effective and that a number of different animals could be used as sources of food supply. Mr. A. L. Ford reared *Diplogaster ærivora* for one year on the macerating bodies of adult *Saperda tridentata* and *Hydrophilus triangularis*. He also found it possible to use macerated beef but the cultures proved somewhat unsatisfactory because of the offensive odor. This nematode was found by Merrill and Ford ('16) parasitizing termites and Mr. Ford has informed the senior writer that he tried the macerating bodies of these insects as a medium but found it

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unusable since a mould almost invariably appeared in the cultures, filling them with threads and obscuring the entire preparation. The writers also tried macerated termites with similar lack of success from the same cause.

Cephalobus dubius.—Maupas ('00, p. 611, 613) found some difficulty in culturing the parthenogenetic nematodes, including this species, stating that "elles se prêtent mal à des cultures en grand." However, the writers have been able to culture *Cephalobus dubius* in countless numbers at any time. Since studies of this species were not begun until cultures of *Diplogaster ærivora* had been in progress for about two years, the methods which had proved most satisfactory were tried for *Cephalobus dubius* and were likewise found to be successful. Several series of cultures were maintained in Pfeffer's solution but many of the life history studies were made using the hen's egg preparation. Both media were used as described in the preceding section, and both proved suitable for prolonged cultures, indications pointing to the hen's egg being slightly preferable. Grasshopper eggs, Colorado potato beetle eggs, and pupæ of Hessian-fly also proved suitable but were not always readily available.

As has been mentioned, other workers made some use of peptone as a nutrient medium for the culture of nematodes. After a number of trials with different strengths of peptone, a solution was found which has proved thoroughly satisfactory and, at the present writing, cultures of *Cephalobus dubius* have been running in this medium for months, without any indication of weakness. The strength of solution used by Potts is not given in his paper. Johnson discovered that strong solutions are inimical to the nematodes and that dilution to as low as 0.15 per cent. was necessary, this strength proving satisfactory for one series of *Rhabditis pellio*. The writers have had a similar experience, finding that strong solutions kill *Cephalobus dubius* in a short time but that very weak ones are quite efficient. In the work on which this paper is based, 0.8–4.6 per cent. solutions were used. In fact, it was found unnecessary to make up solutions of definite strength, except for special studies, since the stock cultures could be kept in flourishing condition by occasionally (every four to six days) adding to the water in the mounting-cell containing the nematodes the small amount of peptone which would adhere to the point of a common dissecting needle. This method of measure was, of course, subject to variation but within its limits the

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varying quantity seemed to produce no appreciable effect on the cultures and indicated a range of over five per cent. within which the solution is suitable. The exact maximum and minimum dilutions for this species were not determined. It should be mentioned in this connection that even longer intervals between "feeding" were possible if necessity demanded. The writers had one peptone culture made up in the above-described way which existed for over four weeks without renewal of the food, the nematodes thriving and reproducing during the whole period.

As stated on a preceding page, Potts found that it was necessary for peptone solutions to putrefy until a cloudy growth of bacteria had developed and that it was "only in the presence of great numbers of bacteria, or the substances formed by them, that the nematodes thrive so well." In cultures of *Cephalobus dubius* maintained by the writers, it was not necessary to develop any putrefaction of the peptone solution but the dry peptone was added directly to the water of the rearing cell. When new cultures were necessary, distilled water was placed in the cell, a small amount of peptone on the end of a dissecting needle was transferred to it, and the nematodes then introduced. This procedure was followed a great many times and without any appreciable diminution in the activities of the animals. Furthermore, cultures made up in this way have been kept for a month without developing cloudiness and the nematodes grew and reproduced rapidly. Since no attempt was made to keep these cultures sterile, bacteria did develop in them to some extent and it may be that in those cultures which remained perfectly clear a limited amount of bacteria was present. It should be mentioned in this connection, however, that a distinct cloudiness did appear in some of the cultures after standing several days, developing gradually into a distinct brown color. This occurred rather commonly in summer and was possibly due, in part at least, to the accumulation of bacteria. But, with this particular nematode, the development of the brown color was accompanied by a general deterioration of the culture and necessitated either a removal and renewal of the greater part of the culture medium or a transferrence of the nematodes to a new solution. It would then seem that *Cephalobus dubius* requires only a very low development of bacteria in the culture medium, if it is required at all, and that an undue development of the cloudiness is usually detrimental.

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General Procedure

Certain general methods of procedure were found to be preferable, and, in some cases, necessary to the maintenance of the cultures. In stock cultures reared in glass mounting-cells, the optimum amount of medium was found to be about two-thirds to three-fourths the capacity of the cell. Most of the cells were kept approximately half filled. Smaller amounts could be used but involved the danger of the cell drying up between observations.

Early in the work, it was noticed that when a stock culture was sealed over completely with a vaselined cover-glass unfavorable conditions became established and the culture would often die out. Experiments were performed to determine the effect of the presence or absence of an air-space above the medium and it was definitely demonstrated that such a space must be provided, hence the above-mentioned procedure of never filling the cells more than three-fourths full. Furthermore, it was found that even with an air-space provided, unfavorable conditions would develop in a few days if the cell was sealed air tight. This situation was avoided by slipping the cover-glass back so that a small crescent-shaped opening between the edge of the cover-glass and the walls of the mounting-cell was produced. This method was entirely satisfactory although it involved a certain loss of the water by evaporation, thus necessitating an occasional restoration to the original level in the culture-cell. The exact reason for this demand for ventilation is not known. Johnson ('13, p. 612), who also discovered the necessity for providing against completely closed cultures, thought that it was necessary to permit the escape of gaseous decomposition products originating in the culture medium. It is true that in cultures made from such materials as yolk of hen's egg, insect eggs, and ovaries of grasshoppers, decomposition products were formed since they tend to quickly develop offensive odors. On the other hand, Martin ('13, pp. 94-97; 143) has pointed out the indispensability of free oxygen to the development of the embryos of nematodes and it may be that the demand for oxygen is the explanation. Since many of the culture media used were undergoing decomposition and since this process draws upon the available oxygen, it is possible that the oxygen supply in one of the sealed culture-cells, having either a small air-space or no air-space at all, would soon be reduced to an unfavorable extent.

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Occasionally, even the partially closed stock cells, especially those containing peptone, developed not only a cloudy appearance but also a brown color which gradually increased in intensity. The specific cause of this color is not known but it was evidently a result or an accompaniment of the general activity of organisms in the cell and almost invariably led to the death of the nematodes if the conditions were allowed to continue. Thus it was necessary either to transfer nematodes to an entirely new culture cell or else remove the old medium and replace it with new. To accomplish the latter, the point of a finely drawn pipette was thrust just below the surface of the medium in the stagnant cell and the liquid very slowly removed. In this way, the eggs, young, and adult nematodes which are always at the bottom were but little disturbed and few of them lost in the process. Then the old medium was replaced with distilled water and a small amount of peptone added. If necessary, a number of the nematodes can be transferred to new culture-cells by means of the transferring needles already described. In stocking a new culture, only a few nematodes need be transferred.

In studies of the reproduction, development, growth, and activities of these nematodes, it was necessary to isolate individuals or eggs in order to follow definitely and accurately the sequence of events. To accomplish this end, cultures of a different type were used. The culture-cell was made up in the same way as those for the stock cultures except that it was necessary to use a cell not over 5 mm. high. A single drop of distilled water from a pipette was centrally placed in the bottom of a clean, dry cell. This drop, if carefully placed, retained its integrity. If the drop spread and came into contact with the edges of the cell, it was discarded. To this drop of water was added a tiny bit of the food material (the quantity which will just cling to the extreme point of a dissecting needle) and the nematode or the egg transferred to it. Since these single-drop cultures have a large air-space compared with the bulk of the fluid and since they must be examined frequently in connection with the recording of data, these cells were kept tightly sealed and in that way the danger of drying up was avoided. During the microscopical examination, the whole cell was removed from the slide, thus leaving the culture drop intact. It was necessary to guard against undue evaporation during the exposure of the drop and all losses had to be replaced. Transference of the nematodes, young or adult, was easily accomplished by means of the needles already described. Eggs were

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transferred by the same means and likewise by the drawn out pipette already described. The latter method was advantageous in transferring a number of eggs at a time since they could be massed together in the culture-cell and then drawn up in the pipette with a relatively small amount of liquid. Furthermore, eggs were transferred by means of the brush described in an earlier paragraph but this method was inferior to the ones discussed above.

Starting Cultures from New Stock

Since *Cephalobus dubius* has been found but once and the entire series of cultures is from the original stock, the writers have had little experience in transferring this species from the conditions of nature to those of the culture-cell. However, *Diplogaster ærivora* has been brought in from outside conditions and established in cultures a number of times. New stock used by the writers usually came from grasshopper eggs and was secured by breaking up the eggs in culture-cells, diluting the yolk with distilled water, covering the cell in such a way as to leave an air-space and a ventilating opening, and putting it aside under temperature conditions of about 68-77° F. Often, after a few days, this nematode would appear in the culture whence it could be transferred to a more dilute medium of the same kind and then later to a different medium, if desired. Mention has already been made of the fact that Merrill and Ford ('16) found this nematode parasitizing termites. Mr. Ford has informed the senior writer that he could almost invariably secure a culture by the following method: A number of termites are killed and placed on the surface of water saturated soil in a small container. After some hours, nematodes usually emerge from the heads of the termites and continue their activities on the moist surfaces of the dead insects. In a few days, the disintegrating bodies are often swarming with the worms. Then a number of them are removed to a culture-cell containing the desired medium. Of those first transferred, the majority may die but usually a few will survive the change and reproduce, starting a new stock. Evidently, in a parasitic form, the transition from the body of the termite to the conditions of a culture-cell is a severe one but the immediate progeny of those which persist flourish in the new medium and thereafter maintenance is simple. This mortality of the parasitic nematodes, when removed to cultures, confirms, in part, the experience of Johnson ('13, p. 613) who frequently had great difficulty in starting cul-

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tures of *Rhabditis pellio*, although, when once started, the difficulties of maintenance were considerably reduced.

Temperature Conditions of Maintenance

While no definite experiments have as yet been made to determine the exact maximum and minimum temperatures for these nematodes, certain observations are worthy of mention here. Temperature records for rooms where some of the cultures were kept showed that both species withstand successfully a considerable variation of temperature. In one room, the daily maximum-minimum records sometimes showed a difference of as much as 33° F. Such variation usually occurred during the winter months when the room temperature occasionally dropped from 74° to 42° F. Under these conditions, daily variation of 15-25° F. were common and a minimum temperature of 40° F. for a limited time (4–6 hours) apparently had no serious effect on the cultures. In this same room during the summer months, the variation was considerably less but both maximum and minimum temperatures were much higher, e.g., 92° and 84° F. It was found that when the room temperature rose much above 80° F., cultures began to show signs of weakness.

In cultures of *Cephalobus dubius*, the nematodes began to die under conditions of 90° F. These cultures were removed to an underground concrete cave where an almost constant temperature of 80° F. was maintained and evidence of strengthening was apparent. During the following thirty days (July), when the temperature in the cave gradually approached 87° F., the cultures showed increasing signs of weakness and ultimately maintenance became difficult. In the following month, when the temperature began to fall, signs of strengthening were evident as soon as 80° F. was approached. When 78° F. was reached, the cultures were soon flourishing again. In other respects, the conditions of rearing were the same throughout this period. Evidently, the maximum temperature for this species is near 90° F. and the optimum below 80° F. At the present time, stock cultures are thriving under laboratory conditions of 65-75° F.

Preliminary studies with reference to the influence of temperature upon *Diplogaster ærivora* were conducted by means of an air conditioning machine having two large breeding chambers in which constant temperatures (within very narrow limits) of 80° and 90° F., respectively, were maintained. These nematodes could not be reared in the 90° F.

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chamber. Cultures submitted to that temperature soon showed decrease of activity, suspended reproduction, and early death. Fresh cultures reared in the 80° F. chamber and transferred to the 90° F. chamber suffered the same fate. Nematodes reared in the 80° F. chamber also exhibited some decrease in activity but continued to live and multiply, producing four generations in forty-three days. Cultures, kept under greenhouse conditions at a mean temperature of about 75° F., increased more rapidly than did those within the 80° F. chamber and the rate of development was greater, five generations being produced in forty-three days. Still other cultures were maintained in the laboratory under a variety of temperature conditions, and, according to the experience of the writers, 65-75° F. is favorable for rearing *Diplogaster ærivora*.

PARTHENOGENESIS IN *CEPHALOBUS DUBIUS* MAUPAS

Distribution

Cephalobus dubius is evidently a cosmopolitan species. Maupas ('00, pp. 555-556) states that it is very common in Algeria and that he found it in a sample of red earth collected in the environs of Tananarivo, Madagascar. In these localities, the nematode lives in rather poor earth and is said to withstand long desiccation and to revive when the moist conditions are re-established. Dr. N. A. Cobb states in a letter that it occurs in various parts of the United States and is found in moist situations, feeding upon animal matter. He also states that on several occasions it has been found on the surface of various insect eggs, and that the young withstand desiccation.

Source of Material

The original stock for all of the writers' cultures was secured accidentally in connection with the study of another nematode. In a preliminary experiment to determine whether *Diplogaster ærivora*, a species found feeding on the contents of grasshopper eggs, has the ability to penetrate insect eggs or whether it depends upon some other agency to provide the means of entrance, ordinary loam, sterilized by steam, was placed in a jar. Grasshopper eggs, cleaned of all soil particles but not subjected to special treatment, were introduced into this soil and specimens of *Diplogaster ærivora* in water added to the adjacent soil. When examined later, the eggs were found broken and deteriorating.

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Under magnification, nematodes were seen in the egg contents and were transferred to culture-cells. It soon became evident that they were unlike *Diplogaster ærivora*, and when submitted to Dr. N. A. Cobb for identification they proved to be *Cephalobus dubius* Maupas. The original source is thus in doubt, but since the soil in the jar had been sterilized, it seems very probable that this nematode was carried into the culture on the surface of the grasshopper eggs, either as an egg or in some subsequent stage of development. Since there is evidence that at least the immature individuals of this species can successfully withstand desiccation, it is possible that they might have occurred on the surface of the insect eggs in that state, resuming activity when introduced into the moist conditions of the experiment.

Parthenogenesis

Maupas ('00), in his extensive study of reproduction in nematodes, found seven species in cultures of which he saw no trace of males. These seven species, distributed among six different genera, included *Cephalobus dubius*. He was inclined to admit the possible occasional occurrence of males but states that if they do appear they must be very rare.

Since the beginning of this work, the writers have watched carefully in the large number of successive generations for any appearance of males but in vain. Mature individuals, isolated in culture-cells, always deposited eggs. Similarly, isolated eggs or young that attained maturity always produced individuals which deposited eggs. Maupas expressed uncertainty as to the constant absence of males because of the fact that, in his studies, the greater part of the apparently parthenogenetic nematodes "se prêtent mal à des cultures en grand" and that with one exception he was unable to examine very large numbers. That uncertainty does not seem so significant since the writers were able, with their methods of culture, to get this nematode in almost any quantity and a very large number was examined for the possible appearance of males. Since the nematodes were, in part, maintained under certain differences and variations of food and temperature, it would seem that if males ever do appear in this species they must be extremely rare and apparently must develop under conditions of culture different in some unknown respect from those of the writers. Cultures of this species are still under observation in order that this matter, among others, may be more thoroughly tested. If it is later discovered that males do occasionally occur,

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it will be interesting to determine whether they have all of the activities and functions of the sex or whether they resemble the imperfect males which occasionally occur in several of the hermaphroditic nematodes.

As Lankester ('17, p. 504) points out, parthenogenesis signifies "an exceptional and historically super-induced modification of the normal process of sexual reproduction or gamogenesis in which the female gamete or egg-cell does not unite with a male gamete or sperm-cell to form a 'zygote,' but proceeds to develop independently." It is a well known fact that protandric hermaphroditism ("Syngonism") is common among the free-living nematodes and Cobb ('15, p. 95; '16, pp. 198–199) suggests the desirability of re-examining the supposedly parthenogenetic forms to determine whether some of them are not actually "syngonic." This suggestion was made on the basis of a study of a series of syngonic free-living nematodes in which the spermatozoa present were smaller and smaller until they reached the optical limits of present instruments and he finds himself unable to assert the non-existence of spermatozoa in certain nematodes merely because he has not succeeded in finding them. This suggests that since both the parthenogenetic and the hermaphroditic nematodes have the unmodified form of the female, the former may, at least in some cases, possibly be instances of masked hermaphroditism.

The writers have not thus far made any microscopical examination of the gonads to determine the above-mentioned point and no comment can be made upon it except that the complete absence of males in continuous cultures maintained for over three years seems to indicate the parthenogenetic method of reproduction. If the animal were hermaphroditic, it would necessitate that every egg produced be fertilized and that fertilized eggs invariably produced hermaphrodites in order for the results of the writers' cultures to have been possible. Furthermore, as Maupas and others have pointed out, the hermaphroditic forms show an unbalanced relation between the number of spermatozoa and ova produced and the unfertilized ova do not develop but disintegrate after they are laid. In the case of *Cephalobus dubius*, all of the eggs laid were capable of development. Also, in the majority, if not in all hermaphroditic nematodes, males appear even though they may in some species be rare and imperfect in their sexual functions. At present, there seem to be no grounds for considering *Cephalobus dubius* other than a parthenogenetic form.

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Oviposition

In order to obtain data on the reproductive capacity of the females, oviposition records were made as follows: Eggs, isolated from vigorous stock cultures, were placed individually in a single drop of distilled water in a glass mounting-cell. Frequent observations were made and the time of hatching carefully noted. A trace of yolk of hen's egg was then added and daily observations continued until the death of each individual. When oviposition began, the number of eggs appearing in the cell each day was recorded and the female transferred to a new culture, thus avoiding the labor of removing the eggs from the cell.

Cultures of thirty-six different individuals, representing a number of successive generations, were carried through the life cycle to cessation of oviposition and subsequent death and showed considerable variation in the length of the egg-laying period. These cultures were all maintained under the same conditions with respect to food but the temperature was that of the laboratory and subject to an average daily variation of about 18° F., the extremes being approximately as follows: minimum, 40-58° F.; maximum, 66-76° F. Temperature was the only factor which varied to any extent and the precise influence of this factor was not determined. Under these conditions, the duration of the egg-laying period was found to have a variation of 6–44 days, the average being about 16 days. This variation seems surprisingly large and leads to the suspicion that those nematodes with a short egg-laying period are individuals which, for some reason, had their normal existence shortened. But, since no account was taken of those individual life histories which were ended at a time when the egg production rate was high and only those which showed an acceleration followed by a subsequent retardation were considered, it does not appear that all such instances can be so interpreted. It should be stated that the maximum oviposition period of 44 days given in this set of records is much higher than any of the others, the next lowest being 31 days.

Egg-laying, when once initiated, continues uninterruptedly until its decline at the end of the life of the individual. None of the records showed any evidence of definite periods of cessation of egg-laying followed by active resumption of oviposition. Only two instances of 24–48 hour periods with no oviposition appears in the records and these occur at the end of the life of the nematode. The eggs are laid singly and at a

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varying rate depending upon the age of the parent. The most characteristic feature of the records on all individuals presenting any evidence at all of a normal life cycle is the rather gradual increase in the daily rate of egg-laying during the first part of the oviposition period and the more or less gradual diminution in the last part. In every case, the maximum daily number of eggs deposited appears well within the oviposition period, ordinarily near the middle. Usually the initial and concluding daily rates are very slow, the latter often ceasing completely before the death of the nematode. In a few individuals only was the initial daily record above 10. The increase and decrease of the daily rate were not regular for any of the individuals studied. The maximum production of 27 eggs in one day occurs in the record of nematode No. 36. A few other records are close rivals. According to Maupas ('00, p. 560), "Par une température de 20° c., le maximum d'œufs pondus, dans les vingt-quatre heures, est de 12 à 13." It is evident that the daily rate of egg production is, at least at times, much higher than Maupas' record would indicate.

The total number of eggs per individual varied from 33 to 285, the average being 139. In general, those nematodes which lived longest and thereby had the greatest oviposition period produced the largest number of eggs but, in the writers' records, no constant relation exists between the duration of the oviposition period and the total number of eggs. For instance, nematode No. 55 produced 101 eggs in 9 days but nematode No. 79 deposited only 77 eggs in 13 days; nematode No. 24 produced 270 eggs in 44 days but nematode No. 85 produced 285 eggs in 30 days. Maupas ('00, pp. 561-562) reported a maximum of 415 eggs deposited over an egg-laying period of about 4 months.

In addition to the above, another set of oviposition records were made from cultures kept in an underground, concrete cave, where, during the period involved, the daily temperature variation was within 5° F., usually within 4° F. The average maximum temperature was 77.8° F., the variation being 75-79° F., and the average minimum temperature was 73° F., the variation being 71-76° F. While the temperature was not completely controlled, the fluctuation was small and the results are of interest when compared with those already described. The remaining conditions of the cultures were like those of the other series with the exception that peptone was used instead of the yolk of hen's egg. Six individuals were

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successfully carried through their complete life cycle and the egg-laying period varied from 15 to 39 days. The total number of eggs deposited by each individual varied from 78 to 234. This series of cultures also presented the characteristic increase and diminution of the daily rate of oviposition which has already been described. The lack of a definite relation between the length of the egg-laying period and the total number of eggs deposited was also apparent although the general tendency for the longer periods to be accompanied by the larger number of eggs was evident. Owing to the difference in the numbers of individuals studied in the two series, a more detailed comparison is not possible.

After oviposition, the development of the embryo goes on rapidly and within a few hours the egg, under magnification, shows signs of internal activity. Records on the development of 88 eggs from deposition to hatching showed 3.3 days as the average egg period, the variation being 2.5-4 days. This is in close agreement with Maupas' statement ('00, p. 561) "les œufs mettent trois jours à parcourir leur embryogènie jusqu'à éclosion."

Immature Period

Length of Larval Stage.—In hen's egg cultures kept under the conditions of the laboratory, the larval period of 48 individuals of different progenies showed a variation of 8-17 days, although the usual variation was 8-14, 17 occurring but once and 14 being the next lowest number. The average larval period, based upon the above-mentioned records, is 10.1 days. In this paper, the larval period is regarded as extending from the moment of hatching to the deposition of the first egg, and not to the cessation of growth as will be discussed later. The appearance of the genital pore is an indication of maturity or closely approaching maturity but it was thought best to use the first oviposition as the final limit of the immature stage. Maupas ('00, p. 561) reported the larval period to be 10-11 days, at a temperature of 68° F. He also used the appearance of the first egg as evidence of the completion of larval existence.

In peptone cultures maintained under the more uniform temperature conditions of the underground cave described previously, the larval period of 10 individuals had a variation of 8-14 days, the average being 10.8 days. It will be noted that this agrees closely with the cultures reared under laboratory conditions and it appears that limited differences of temperature and the comparative culture values of peptone and yolk

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of hen's egg had no striking effect on the time interval demanded for the attainment of sexual maturity.

Rate of Growth.—Careful, daily measurements of length of 8 individuals reared in peptone cultures under the cave conditions were made for about 22 days, a period of time well beyond maturity and the cessation of growth. It was noted that the period of growth and the larval period are not coterminal, but that growth usually continues 3-6 days after the production of the first egg. During this time, the increase in body-length has a variation of 0.019-0.076 mm., the average being 0.0527 mm.—about 10 per cent. of the average body-length of the completely formed individual.

At hatching, the larva is only 0.140-0.179 mm. long, the average of 16 specimens being 0.162 mm., and, from that time to the attainment of the full-grown condition, growth is continuous and approximately uniform. The growth curves of different individuals are very similar. The average daily increase in length was found to be 0.0258 mm., the variation being 0.019-0.03 mm. There is no evidence of a definite maximum daily rate of growth anywhere in the period and apparently growth, when completed, ceases almost as abruptly as it begins. The average growth period was approximately 16.3 days, with a variation of 14-18 days.

Length of Life

The complete life showed a variation of 20-61 days. Only 1 nematode lived 61 days, but 8 of 29 individuals lived 40-50 days, and 11 lived 30-40 days. The variation indicated above appears to be rather large and may have been due to some peculiar combination of factors in the different cultures, although all were given the same kind of food in approximately the same amount and kept under the conditions of the laboratory. Maupas ('00, pp. 561-562) records one individual which lived for approximately five months. The egg-laying period has already been discussed and it thus appears that if the first eggs of the parent be considered, a new generation can be produced in the cultures every 11-19 days, or about two generations per month.

REPRODUCTION IN DIPLOGASTER ÆRIVORA COBB

Source of Material

This nematode was first discovered in 1914 infesting the eggs of grasshoppers after they had been deposited in the ground. Specimens

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were sent to Dr. N. A. Cobb who reported them as representing a new species. Later, Merrill and Ford ('16) found nematodes infesting the termite, *Leucotermes lucifugus*, which, when submitted to Cobb, proved to be the same species as the one found in grasshopper eggs and he described it under the name *Diplogaster ærivora*. Merrill and Ford made a special study of this nematode in relation to the termite host and published, in addition to Cobb's original description of the species, certain data on the reproduction and habits of this worm as observed in cultures. The present account includes the results of a more prolonged study of certain features of the reproduction, based upon continuous culture studies of more than three years.

Oviparity

Merrill and Ford ('16) have already pointed out the fact that males and females are continually present and that the males are functional. Cobb, in the same paper, described the morphological features of the two sexes. The writers have also found this species reproducing exclusively by the bisexual method. No evidence of hermaphroditism or parthenogenesis appeared, although evidence of such phenomena was sought continually.

Copulation

Copulation was easily studied in the cultures and the observations of the writers essentially confirm the brief account of Merrill and Ford ('16, pp. 125-126). Increased activity on the part of the male before mating was noticed. A female will copulate with several different males in a short time and the same is true of the behavior of the male towards different females. As will be shown later, observations indicate that a female must mate two or more times in order to produce fertile eggs throughout the adult life.

Fertile Eggs

The normal, fertile egg of *Diplogaster ærivora* is oblong, the average dimensions from forty-five measurements being 0.064 mm. and 0.035 mm., the variation being within 0.007 mm. The outer covering consists of a tough, membranous coat. Eggs are always deposited singly.

Infertile Eggs

An egg, which has seemingly never been fertilized and from which a nematode never develops, is designated as an infertile egg in these

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studies. Such eggs are larger than the fertile ones, their dimensions being about 0.084 mm. and 0.043 mm. They are slightly variable in shape but are usually suboval. The envelope consists of a very thin membrane and encloses the finely granular, light colored contents. The general appearance of such eggs is sufficiently different from that of the fertile eggs to make it comparatively easy, with a little practice, to recognize them at sight in the cultures.

Relation of Copulation to Egg Production

An interesting relation between copulation and the production of both fertile and infertile eggs exists in this species. Fertile eggs were never deposited by a female previous to the initial copulation. A female, upon being mated, will, for a time, lay fertile eggs after which infertile ones will be deposited until another mating occurs. Upon being mated the second time, the female will again produce fertile eggs. For example, female No. 12 was mated at maturity, after which the male was removed from the cell. Twelve fertile eggs were deposited by this individual during the next 48 hours. During the following 60 hours, 8 eggs were extruded, all of which were infertile. After 6 days, this female was mated a second time and as a result, within 3 hours after copulation, fertile eggs were again deposited. Fifteen fertile eggs were laid during the following 3 days and 2 fertile eggs were found in the body of the female at death. Essentially, the same results were obtained in several different individuals maintained under similar conditions. On the other hand, female No. 88 was kept constantly exposed to males throughout her mature life and fertile eggs were uninterruptedly deposited until her decease. After the first mating of this female, eggs soon appeared and the number rapidly increased until well towards the end of the 2nd day at which time the maximum deposition of 19 eggs occurred. From this time on, there was a gradual reduction in the number of eggs produced until cessation occurred at the end of the 6th day, death ensuing about 24 hours later.

Occasionally, a female would completely exhaust her ability to deposit fertile eggs before any infertile ones were produced but, in the majority of cases, the approaching cessation of fertile egg production was indicated–not only by the reduction in the number but also in the appearance of a mixture of fertile and infertile eggs leading to the final disappearance of the former. After a second or subsequent mating, the same phenomenon

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usually occurred except in the reverse order, although, in some cases, there was an abrupt cessation of the infertile egg production after re-mating the female. Commonly, the maximum egg production follows the first mating, subsequent ones being followed by the deposition of a smaller number of eggs over a shorter length of time, but this is not a constant feature since a few exceptions appear in the records, as for example, female No. 81 was mated 3 times within a period of 11 days and the maximum production followed the third mating.

It thus appears that in order for the female of *Diplogaster œrivora* to attain her complete reproductive capacity, she must be mated at intervals throughout the egg producing period. One mating is insufficient but the reasons for this insufficiency are not definitely known at present. In all of the matings, the period of production of fertile eggs never exceeded 3 days and it may be that this represents the extent of the life of the spermatozoa after they have been transferred to the female. There is no reason for believing that the fertilization is other than *hysterogamic* (Lankester, '17, p. 505) and possibly fertile eggs cease to appear when all the spermatozoa of a single mating have been exhausted, but, if this be the case, there must be considerable variation in the number of spermatozoa transferred to the female at copulation since, according to the writers' records, the number of fertile eggs resulting from a single mating varied widely, as for example, initial matings resulted in 7-113 fertile eggs. Possibly the length of copulation, which is known to vary in this species, determines, in part at least, the number of male cells transferred.

Maupas ('00, pp. 586-587; 601-602) pointed out that there is a striking imperfection in the protandric hermaphroditic nematodes in which fertile eggs will be produced until the supply of spermatozoa is exhausted and then the same nematode will continue to produce infertile eggs in numbers 2 or 3 times greater than the fertile ones. The evidence seems to support the contention that this condition is the result of the failure of the ovo-testis to develop enough male cells. The infertile eggs invariably deteriorate. He also found that the occasional males of these hermaphroditic species sometimes fertilized the hermaphroditic individual after it had exhausted its own supply of spermatozoa. These cases may be comparable, if not homologous, to the condition in *Diplogaster œrivora*. Certainly, the disadvantage to the species is similar. Since all of the infertile eggs of *Diplogaster œrivora* deteriorate, the reproductive capacity of a single female is limited greatly unless males are ever present to fecundate her.

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Viviparity

At times, another form of reproduction occurs in the life history of this nematode in which living young appear within the body of the mother. This phenomenon existed many times in the cultures and was carefully studied. The first indication of this change in the normal reproductive procedure is the appearance of one or more very tiny nematodes within the body of the parent and inside of the uterus. These young move about actively and increase in size at a rapid rate. In time, they break their way through the wall of the uterus into the body-cavity of the mother and begin to actively attack her internal organs, soon causing her death. The young continue to feed upon the body contents of the dead parent until, in many cases, the whole interior is hollowed out, leaving nothing but the transparent cuticula within which the developing young wriggle about. From this empty parental cuticula, the young escape to the exterior and take up an independent existence in the surrounding nutrient medium. Approximately one-third to two-fifths of the complete growth may be attained within the parent. Merrill and Ford ('16, p. 126) observed this phenomenon in the parasitic strain of this species which they studied and they state that "Usually they were unable to escape, although instances were observed where they escaped through the genital pore of the mother." They also figure a dead female containing 14 young, all of about the same degree of development. It might be inferred from the above quotation that few of these young are able to complete their development, but the long observations on which this paper is based show that, in the strain from the grasshopper eggs, not only is the phenomenon fairly common but that the large majority of the young so developed escapes through the genital opening of the mother or through some rupture of her body-wall. Not only do such young complete their growth but they are perfect individuals and capable of reproduction. The number of young developing within the parent is variable. The writers have records of as many as 20 appearing at about the same time and also some evidence that even more may be so produced. On the other hand, the number may be as low as 3 or 4. These larvæ develop into both males and females and, since living young were never observed in females which had not at some time been exposed to males, it appears that they arise from fertilized ova and not from parthenogenetic ones.

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Reproduction of this viviparous sort has been observed before in nematodes. Maupas ('00) found it to be a common occurrence in *Rhabditis elegans*, *Rhabditis caussaneli*, and *Diplogaster robustus* in which the eggs are not deposited as rapidly as they arrive in the uterus but tend to accumulate there, the delay causing them to be deposited ultimately in an advanced stage of development. Some of the young hatch in the uterus and are expelled along with the unhatched eggs but when the supply of spermatozoa is exhausted and the infertile eggs pass into the uterus, the young hatch, feed upon the infertile eggs accumulating there, grow, rupture the wall of the uterus, scatter in the general cavity of the mother, disorganize and devour the internal parts, and ultimately escape to the exterior. Pérez (Conte, '00b, p. 375) observed viviparity in *Rhabditis teres*, Conte ('00a; '00b) recorded it in *Rhabditis monohystera* and *Diplogaster longicauda*, and Southern ('09, pp. 93-94) described the occasional appearance of young within the body of the mother in *Rhabditis brassicæ*. Merrill and Ford ('16, p. 120) apparently found a similar occurrence in *Diplogaster labiata* in which "Occasionally a young nematode hatched within the body of a dead female," but no statement is made as to the ultimate fate of such individuals.

The factors initiating and influencing the appearance of this viviparity are not definitely understood. Conte ('00a) found that *Rhabditis monohystera "vivipare sur colle de pâte, est ovipare sur peptone"* and that the conditions of development are influenced by the nature of the nutritive medium. In another paper, Conte ('00b) points out that Maupas ('99) attributes the appearance of this viviparity to two causes, inanition and senility. Conte, however, found evidence that putrefaction in the medium was a cause, at least in the case of *Rhabditis monohystera*. "Tout en admettant avec lui que, dans certaines espèces, l'inanition et la sénilité amènent le parasitisme embryonnaire, je crois que ce phénomène peut être provoqué par d'autres causes et notamment, ches *Rhabditis monohystera*, par la putrèfaction du milieu. D'une façon générale, je pense qu'il est en relation avec un état morbide de la mère." In connection with his studies of the production of eggs or young in these nematodes, he finds it possible to distinguish the following stages which are closely related to the character of the nutritition:

Absolute oviparity—deposition of unsegmented eggs.

Relative oviparity—deposition of eggs undergoing segmentation.

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Ovo-viviparity—deposition of gegs containing active embryos.
Viviparity—deposition of young which hatched in the uterus of the mother.
Embryonic parasitism—consumption of morbid mother by her offspring.

There is certainly a tendency in *Diplogaster ærivora* for this form of reproduction to appear towards the end of the reproductive period and while the parent may live and show body movements for a brief period after young appear within her body, her reduced vitality is apparent and is an accompanying feature, if not the causative one, of this phenomenon. However, the writers have evidence that age may not always be an accompanying factor, in fact, there is circumstantial evidence that any set of conditions which interferes seriously with the well-being of the female may lead to the appearance of living young within her body, even early in the reproductive period. It is therefore the opinion of the writers that this viviparity is the result of reduced vitality of the mother rendering her incapable of discharging the eggs. It also appears that eggs formed early in the reproductive period may hatch within the body as well as those produced at the end, and the writers have thus far discovered no positive evidence of any inherent predisposition of the last formed eggs for internal hatching.

There is no question that this appearance of young within the body of the mother constitutes a form of reproduction and that the resulting offspring are just as capable of continuing the species as those arising from hatching outside of the body. In reality, these two forms of reproduction are only superficially distinct and the use of the terms *oviparous*, *ovo-viviparous*, and *viviparous* is mainly one of convenience rather than one of exact distinction. Lankester ('17, p. 505) holds that "really all animals are viviparous, for the birth-product is a living thing whether it is a naked egg-cell or more or less advanced in development. The enclosure of the birth-product is a shell or case, which has given rise to the term 'oviparous' is not of any value as indicating the real degree of development of the young at birth, for in some cases unfertilized egg-cells, in others mere discs of developing embryonic cells (as in birds, etc.), and in yet other cases well-shaped young ranging from the early larva of some invertebrates up to the completely formed miniature of the adult, as in some of the shell-bearing snails, may be enclosed within an egg shell when 'laid' by the mother. There is accordingly no *general* importance

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to be attached to the distinction between 'viviparous' and 'oviparous' animals." In this paper, these terms have been retained for the sake of convenience.

Proportion of Sexes

In the cultures studied by the writers, the total egg production of females varied widely, the average being about 55 eggs per female. The maximum observed was 196. In an attempt to determine the proportion of the sexes and the number of eggs and young per female, eggs selected at random were isolated, each in a separate culture. Females resulting from these eggs were mated at regular intervals throughout their lives. A complete daily record was kept of all eggs laid by these females and the sex of the resulting young determined by rearing them to maturity. Twenty-two females were studied in this way and, of the 437 resulting offspring, 182 developed into males and 291 became females. Since it was not possible to eliminate a certain mortality among the eggs and larvæ, the numbers given above can be regarded only as a general indication. They do show, however, that, in contrast to some of the bisexual species, the males are very common although the females are in the majority. These results agree, in general, with those of Merrill and Ford ('16, p. 126).

Records from the progeny of 22 females show that in the vast majority of cases both males and females appear in each progeny and also that in most of the progenies, females were numerically dominant. Since all the evidence indicates that the species is exclusively bisexual in its mode of reproduction, a sufficient number of males is demanded to maintain the generative processes, but since it was observed that a single male may and often does copulate with a considerable number of females, it seems probable that the smaller number of males indicates no important disadvantage in the multiplication of the species. In fact, the numerical dominance of the females coupled with fewer but sexually active males may possibly facilitate the production of a larger number of offspring, even if the locomotor ability is poorly developed.

Cobb ('18, p. 477), in discussing the comparative rarity of males in free-living nematodes, states that "There is reason to think that in some of the species the males are short-lived, and that this is the reason they are so rarely seen. The males are often so much smaller than the females that they are easily overlooked, or mistaken for young, so that in

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such cases the rarity of the males may easily be overestimated." Since the length of life of the males and females of *Diplogaster œrivora* is virtually the same and since the length of the female exceeds the length of the male by only about one-fifth, it would seem that these features have not affected the observations on this species.

Rate of Growth

The young nematode, upon emerging from the egg, is active and moves about in much the same way as the adult. Its average length at emergence is about 0.238 mm. and the average diameter about 0.015 mm. A number of specimens were carefully studied in individual cultures for the rate of growth, all being reared under the same conditions and all living 12-23 days after hatching. Careful measurements were made at the time of emergence from the egg and at regular intervals of 24 hours thereafter throughout the life of each individual. Data were secured from 4 males and 5 females which were carried through their entire existence, each manifesting all of the activities of a normal individual and living for some time after growth had ceased. From the sedata, growth curves were constructed for the increase in length and diameter. These curves showed a striking similarity, not only in the different individuals of the same sex, but also in the individuals of the different sexes. Furthermore, the curves for the increase in length and the increase in diameter showed very close correspondence in every case. Growth begins immediately at hatching and continues uninterruptedly for a period, after which it ceases permanently. Composite graphs constructed on the basis of the individual growth curves showed that growth in both length and diameter ceases, on the average, on the 8th-9th day after hatching. In all cases except one, the variation from this average was very slight, the exception showing no growth after the 4th day. The average length of the males at the end of the growth period was 0.864 mm. and the diameter 0.052 mm., while the length of the females was 1.105 mm. and the diameter 0.068 mm. The length of life of the adult following cessation of growth varied with the individual from 6 to 15 days inclusive.

Length of Life

The length of the life of this nematode is, no doubt, subject to variation under different conditions. One series of eggs kept under average

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temperature conditions of about 75° F., variation within 7°, required an average of 17.9 hours from the time of oviposition to hatching, the variation being 17-20 hours. A series of 29 individuals showed an average larval period of 3.75 days, the variation being 1.3-8 days. Twenty-three individuals of the same series had an average adult life of 13 days, the variation being 5-21 days. The average life of the individual outside of the egg was found to be about 17.1 days. Including the egg stage, the average life was approximately 18 days. Assuming copulation on reaching maturity, a generation can be secured in about 4.5-5 days. But very little difference was observed in the length of life of males and females.

SUMMARY

Methods

1. Some of the free-living and semi-parasitic nematodes can be reared generation after generation in artificial media and their study thus facilitated. Two species, *Cephalobus dubius* Maupas and *Diplogaster ærivora* Cobb, were cultured continuously for over three years.

2. Cylindrical glass mounting-cells sealed with pure vaseline to ordinary microscope slides and covered with vaselined cover-glasses were found to be the most suitable containers for cultures. Methods of transference are described.

3. Of the numerous substances used as media, a very dilute solution of peptone, diluted yolk of hen's egg, and Pfeffer's synthetic agar were most extensively used. The eggs, ovaries, and body tissues of a number of insects were found to be favorable when properly prepared.

4. Unfavorable cultural conditions developed in stock cultures which were tightly sealed. Provision for ventilation was necessitated.

5. In starting cultures of *Diplogaster ærivora* from new stock taken from nature, mortality in the first generation was high but usually a few survived in the new medium and subsequent maintenance then became simple.

6. Temperatures above 80° F. are unfavorable, 90° F. and above proving fatal. These nematodes withstand a considerable fluctuation of temperature, e.g., 32° F. in 24 hours. Temperatures as low as 40° F. can be withstood, at least for a limited time. Optimum temperature conditions seem to be near 65-75° F.

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CEPHALOBUS DUBIUS

1. Observations on long continued cultures of *Cephalobus dubius* involving many individuals and generations revealed no traces of males and reproduction seems undoubtedly parthenogenetic. If males ever appear, they must be extremely rare and develop under conditions of culture different in some unknown respect from those employed by the writers.

2. The egg-laying period has a variation of 6-44 days, average about 16 days. Oviposition, once initiated, continues uninterruptedly. The daily rate increases somewhat gradually to the middle of the period and then declines. As many as 27 eggs per day may be deposited. The total number of eggs per individual showed a variation of 33-285, average 139. Apparently, all eggs were capable of normal development.

3. Under cultural conditions, larvæ emerge from eggs 2.5-4 days after oviposition and reach maturity in 8-14 days.

4. The growth period and the larval period are not coterminal, growth usually ceasing 3-6 days after sexual maturity, during which time an increase in body-size of about 10 per cent. occurs. Larval growth is continuous and approximately uniform. The average daily increase in body-length is about 0.026 mm. Growth curves of different individuals are very similar.

5. The length of life has a variation of 20-61 days. Computing from the first eggs of a parent, a new generation can be secured in cultures every 11-19 days.

DIPLOGASTER ÆRIVORA

1. *Diplogaster ærivora* is bisexual and males are completely functional. One female may copulate with several males and males may behave similarly towards different females.

2. Both fertile and infertile eggs are deposited. Fertile eggs follow mating for a time, after which infertile eggs are laid until a second mating, after which the same sequence usually occurs. Constant exposure to males may completely prevent deposition of infertile eggs. Approaching cessation of fertile egg production is often indicated by the appearance of a mixture of fertile and infertile eggs. Usually, maximum oviposition follows the first mating.

3. All of the evidence indicates that the female must be mated at intervals throughout the egg producing period in order to fulfil her com-

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plete reproductive capacity. The insufficiency of one mating is not definitely understood but it seems probable that infertile eggs appear upon the exhaustion of the spermatozoa received from a single mating. A similar imperfection in the reproductive ability appears in the protandric hermaphroditic nematodes and is explained by Maupas and others as due to exhaustion of the limited supply of spermatozoa.

4. Viviparity occurs from time to time. Young appear, first within the uterus, then later within the body-cavity of the mother, feeding upon her internal organs, untimately causing her death. In many cases, the interior is completely consumed, leaving nothing but the transparent parental cuticula from which the young escape to take up independent existence in the surrounding medium. Young so produced evidently originate from fertilized eggs and develop into both males and females capable of normal reproduction.

5. This form of viviparity has been observed by other workers and certain factors have been proposed as being responsible for the appearance of this phenomenon, namely, inanition and senility (Maupas), and putrefaction of the medium (Conte). The writers have incomplete evidence that it is the result of any factor or group of factors which reduce the vitality of the mother. Definite evidence was secured to show that, at least in *Diplogaster ærivora*, age is not always an accompanying feature but viviparity may appear at any time during the egg-laying period.

6. Females are numerically dominant, both in mass populations and in single progenies. Males, however, are common and very rarely absent, even in a single generation. Although fertilization is evidently necessary for any reproduction, it is probable that the smaller number of males is compensated for by their constant presence and their ability to copulate with several females.

7. Growth curves based upon daily measurements of length and diameter are strikingly similar for all individuals, irrespective of sex. Composite graphs showed the cessation of growth occurred on the 8th-9th day. The length of life following cessation of growth has a variation of 6-15 days..

8. The average length of life under cultural conditions is about 18 days. A new generation can be secured every 4.5-5 days. No marked difference in the length of life of males and females was observed.

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A NEW AND REMARKABLE DIATOM-EATING FLAGELLATE, *JENNINGSIA DIATOMOPHAGA* NOV. GEN., NOV. SPEC.

ASA. A. SCHAEFFER

Diagnosis. Shape, cylindrical, 180 microns long by 40 microns in diameter; very metabolic. Flagellum, large, 150 microns long. Cuticula with spiral striations 1 to 2 microns apart; numerous movable club shaped appendages about one and one-half microns long on the striations. Large central nucleus, 35 microns in diameter. Large contractile vacuole near the anterior end. Several rod-like structures in the pharynx immediately internal to a large mouth at the anterior end. Numerous bodies in the form of rings or strongly biconcave discs from 1 to 3 microns in diameter, in the endoplasm; large clear spheres up to 6 microns in diameter sometimes present. Locomotion, creeping. Food, exclusively diatoms. Reproduction, asexually, by longitudinal fission. Habitat, marshes, among algae and diatoms.

This remarkable flagellate has come under my observation on three different occasions: February 27 and March 31, 1916, and March 5, 1918. In the three instances, material was collected from the Lonsdale marshes near Knoxville, and allowed to stand in vessels in the laboratory for several months. The organism may be considered rare, for frequent examinations of material from these marshes during the past five or six years has revealed its presence only three times.

This flagellate does not occur in large numbers, not more than a few hundred being found at any one time. And their length of life in the culture is as short as their number is small. In none of the three cases mentioned did they remain in the culture for more than a week. Its rarity, and the difficulty with which it may be cultured are unfortunate from the point of view of the biologist, for its predatory animal nature stands out in striking contrast to the plant-like character of some of its immediate relatives.

I propose for this organism the generic name *Jenningsia*, in honor of my friend Professor H. S. Jennings, and the specific name *diatomophaga*.

Jenningsia is one of the largest members of the flagellates. Although the average length is about 180 microns, several individuals were found which measured 260 microns, exclusive of the flagellum, which in these individuals measured 170 microns, and in the smaller individuals, 150 microns. The shape of the body is cylindrical, with slightly tapering anterior end and very blunt but also slightly tapering posterior end. The

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flagellum is very stout and during locomotion is directed straight ahead, only the apical part being usually in active motion (figs. 1, 2).

In the general character of its movements this organism resembles peranema. Locomotion is accomplished only by creeping, the organism being incapable of swimming. Violent metabolic movements accompany locomotion especially when an obstacle is encountered in its path. Only rarely is the organism stretched out straight. When the direction of locomotion is changed or when a strong stimulus is received from another organism the shape assumed during locomotion is entirely lost in a very violent twisting and kneading movement, which soon, however, gives way again to the cylindrical shape characteristic of locomotion. A very slight stimulus is sufficient to cause very marked metabolic movements.

The exact manner in which locomotion is brought about could not be ascertained satisfactorily, but it was observed that the tip of the flagellum was habitually bent in the form of a small loop which was used somewhat in the manner of a paddle so as to pull the organism along. During locomotion, it is worthy of note, the organism rolls over frequently though not regularly, differing in this respect from the peranemas.

Externally, *Jenningsia* is radially symmetrical like the euglenas. The body is covered with a thin cuticle which is marked with spiral striations that take their origin in the mouth. Small slender spindle-shaped structures about one and one-half microns in length, which may readily swing about in the water, are attached to the striations at irregular intervals. These cuticular spindles are most numerous near the extreme anterior end.

Internally there are found: a large central oval nucleus about 35 microns in diameter with a central denser body about 15 microns in diameter; a complicated contractile vacuole system (similar to that found in euglenas) near the anterior end, which functions several times a minute; a complicated pharynx provided with stiff rod-like elements; and numerous strongly biconcave discs or rings of clear bluish green material, ranging from one to three microns in diameter. One or more large vacuoles are also occasionally found in the posterior half of the organism.

Of the structures just enumerated the pharynx is of especial interest although it is very difficult to understand its detailed operation while feeding. In the moving organism one can see two rod-like elements lying with their anterior ends in a clear space free from protoplasm and

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protected by a strong arched structure on the anterior side, the arch representing the lips of the closed mouth. When the animal is compressed under the cover glass the mouth is forced open and three large rods are now seen which are forced partly outwards (fig. 3). In this process some of the small rod-like elements disappear, which may indicate that these apparent rods are only folds of cuticle occasioned by the closure of the mouth and the retraction of the pharynx. Although I have seen the animal devour diatoms on several occasions I have been quite unable to determine exactly how the rods of the pharynx operate during this process.

Another element of interest are the numerous rings or discs scattered throughout the endoplasm (figs. 1, 4). They are of all sizes as stated above and are of a clear but pale bluish green color. They are unaffected by the ordinary stains or iodine but dissolve without visible change in solutions of H_2SO_4. In their general appearance, number, and behavior towards reagents they are similar to the crystals in amebas and may possibly be formed in a similar manner. They are not active under the polariscope. It is probable that these rings or discs are some by-product of metabolism.

In one culture of few but large individuals, numerous spherical bodies were observed which stained deeply with haematein. No food was observed in any of these individuals.

The most interesting thing about this organism from a general point of view is its mode of nutrition. It is completely holozoic. As indicated by its proposed specific name, its food is living diatoms of all sizes up to 100 microns in length. I have examined in all several hundred of these organisms and nearly all of them had from one to five diatoms, mostly of medium size, in them. No other food objects have been observed.

The feeding process is very difficult to observe in detail, owing to the violent metabolic movements, the activity of the complicated pharynx, and the speed with which the food is devoured. The organism moves along with the tip of the flagellum moving about until it comes into contact with a diatom. If hungry, the flagellum is brought into contact with the diatom as much as possible while the animal continues with its forward movement. When the anterior end of the organism comes nearly into contact with the diatom, the posterior end rears up and violent metabolic movements set in. The anterior end is brought over the

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diatom and is seen to spread out. The basal part of the flagellum is also seen to move about as if it had a part in the actual swallowing of the food particle. Presently the diatom is seen inside the flagellate, which moves away within a few seconds, the whole process of feeding taking place within about 20 seconds. Although I saw a number of instances of feeding and took particular pains to see whether the pharyngeal rods were actually protruded, or were used merely in distending the mouth, I was unable to determine their exact function. I incline to think however, that the rods were not protruded beyond the mouth opening. On several occasions I tested their reactions to carmine particles without obtaining positive responses, although one specimen so tested later devoured a diatom. All the evidence therefore indicates that *Jenningsia* is a predacious animal that feeds exclusively on living diatoms.

I observed but one instance of reproduction—asexual—which was accomplished by longitudinal splitting, beginning at the anterior end and extending backwards. One of the daughters inherits the old flagellum, the other grows a new one during division.

Jenningsia affords a very good example of the ease with which fundamental instincts and habits may be changed in phylogeny, for it is an animal descended from plants. The exclusively predacious instincts of this animal contrast strongly with the true holophytic mode of nutrition of some of the closely related family of euglenas. In so far as the actual process of feeding is concerned we have exhibited within the span of the single order Euglenineae all the general methods of nutrition known among nucleated organisms: true holophytic nutrition by means of chlorophyll among euglenas; saprophytic, the ingestion of decomposing nitrogenous foods either liquid (astasias) or solid (peranemas); holozoic, ingestion of carbohydrate or protein materials—pieces of animal or vegetal tissues (peranema); holozoic, ingestion of small masses of bacteria or pieces of animal and vegetal tissues and on living protophyta (dinema); holozoic, predacious, injestion only of living, moving organisms that, in a real sense, have to be captured (Jenningsia). No better illustration than this could be found of the combined plant and animal characters of the Flagellata.

The change from a holophytic to a holozoic type of nutrition in the Euglenineae has been made possible by the development of the pharynx. In the euglenas the pharynx is a simple tube-like depression at the anterior end whose chief function seems to be that of an efferent drainage

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canal for the contractile vacuole. But it also serves as a means of taking into the body small solid particles as has been shown by placing carmine in the culture fluid. The taking in of solid matter is however not an essential function for the euglena, but it is of interest in that it foreshadows saprophytic and holozoic instincts and modes of nutrition in the so-called colorless euglenas—the astasias—and in the peranemas.

The astasias have gone a step further than the euglenas. They have developed a larger pharynx and, we may suppose, one better adapted to taking in liquid food. At the same time they have also lost the chlorophyll from their bodies.

The peranemas have likewise gone a step further than the astasias in the development of the pharynx. In these organisms the pharynx is provided with special rods which make it possible to open and close the pharynx, and also to act somewhat like a suction apparatus by means of which solid matter may be eaten with despatch. Most of these organisms are small, so that they are restricted in their food to bacteria or small pieces of disintegrating organisms. In Jenningsia we have however as a most important development, a large size, so that it is possible for it to feed on such large actively moving organisms as diatoms in a truly predatory manner.

In company therefore with a progressively developing tendency in these organisms from holophytic to holozoic nutrition in a physiological sense, we have also, on the morphological side, a progressive development of the pharynx and of the size of the organism which makes possible the capturing and eating of relatively large masses of solid food and actively moving organisms.

University of Tenn.,
Knoxville, Tenn.

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EXPLANATION OF PLATE

Fig. 1. *Jenningsia diatomophaga* in locomotion. Body length, 180 microns, flagellum length, 150 microns, cv, contractile vacuole; d, ingested diatoms; e, excretion bodies; m; mouth; n, nucleus; p, pharynx; r, pharyngeal rods; v, vacuole.

Fig. 2. Photomicrograph of living *J. diatomophaga* in locomotion. The dark masses represent ingested diatoms. The basal part of the flagellum is seen at the anterior end.

Fig. 3. Sketch of anterior end compressed under cover glass to force open the mouth, m. s, cuticular striations originating in the mouth and running spirally around the organism; r, the pharyngeal rods.

Fig. 4. Enlarged view of "excretion" discs. a, top view; b, cross section.

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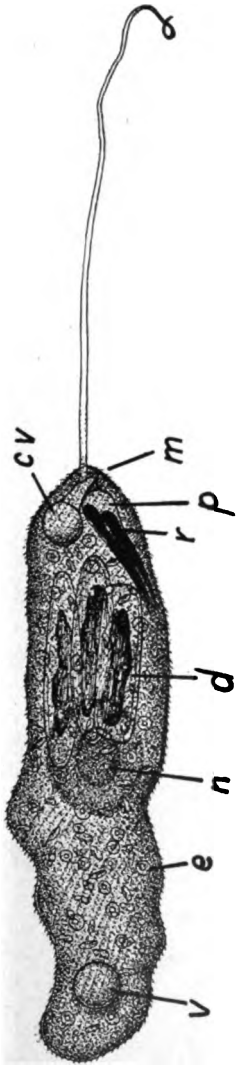

FIG. 1

FIG. 2

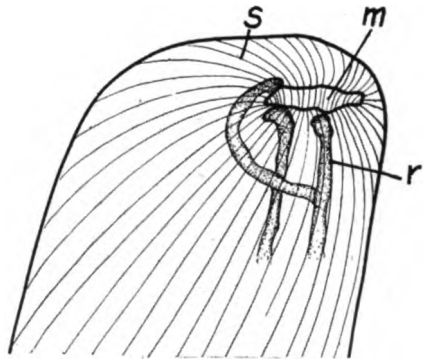

FIG. 3

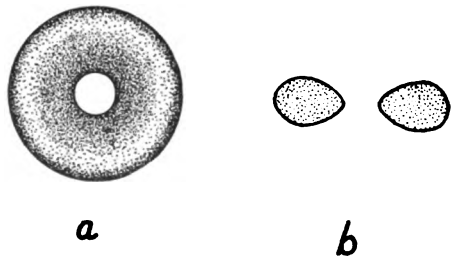

FIG. 4

PLATE XIII

SCHAEFFER

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STUDIES ON AMERICAN STEPHANOPHIALINAE*

With Two Plates

ERNEST CARROLL FAUST

Since the time of O. F. Müller helminthologists have recognized certain distomes which are characterized by a coronet of papillae around the oral opening. These were first classed as Armata, which group included also all echinostome species. The more modern studies of Looss and Odhner have shown that certain of these species are related to the Allocreadiidae. With *Bunodera luciopercae* (*nodulosa*) as the type Looss (1902:453) proposed the subfamily Bunoderinae for the then known forms, *Crepidostomum metoecus*, *C. farionis* (*laureatum*), *Distomum petalosum* and *Bunodera luciopercae*. However, the enormous extent of the uterus crowding the entire posterior part of the body in the case of Bunodera and the scanty coiling of the uterus entirely anterior to the anterior testis in the case of the other species constitutes so marked a difference that the latter species were necessarily removed from the Bunoderinae. Odhner (1905, 1910) placed them in the Allocreadiinae. Nicoll (1909) showed that they were more closely related to the Allocreadiinae than to the Bunoderinae, but that certain differences possessed by the entire group justified the creation of a new subfamily, Stephanophialinae. The writer's study of the American representatives of this group stands in support of Nicoll's thesis, and contributes certain data which mark out this subfamily more definitely.

The following description supplements Nicoll's original diagnosis. The integument is usually aspinose, but in the case of a few representatives of *Stephaniphiala farionis* (fig. 3) spines are found around the oral sucker. The circumoral papillae are always six in number, altho there is a tendency for the dorsal papillae of Crepidostomum spp. to bifurcate. The yolk glands in this subfamily extend from the region of the pharynx to the posterior end of the body. Moreover, the excretory bladder extends to the anterior border of the anterior testis, whereas in the Bunoderinae it is a small pouch posterior to the testes. In the Allocreadiinae as at present constituted there is no common type of excretory bladder. In the genus Allocreadium it terminates behind the anterior testis.

*Contributions from the Zoological Laboratory of the University of Illinois No. 115.

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There are recognizable in the subfamily Stephanophialinae three genera, Stephanophiala, Crepidostomum and Acrolichanus. Stephanophiala contains the cosmopolitan species, *S. farionis* (*laureatum*), and a new species, *S. vitelloba*. Crepidostomum contains the type, *C. metoecus*, *C. cornutum* and a new species *C. illinoiense*. Acrolichanus is monotypic, being represented by *A. petalosa*. The type species of the Bunoderinae, *B. luciopercae*, has been found in the North American perch, *Perca flavescens*, by Stafford (1904:489) and also by Lander.

The writer is indebted to Professor Henry B. Ward for valuable data and material, and to Professor Edwin Linton, Professor Henry L. Osborn, Dr. Arthur R. Cooper and the Bureau of Animal Industry of Washington for the loan of material.

Stephanophiala Nicoll 1909

This genus was created by Nicoll in 1909 to separate *Crepidostomum farionis* of Müller (*laureatum* Zeder) from *C. metoecus*. The separation was made on the basis of 1) disposition and number of the oral papillae, 2) position of the genital pore, 3) size of the cirrus pouch, and 4) extent of the uterus with size and number of the ova. These are the characters which require that *Crepidostomum farionis* be placed in a separate genus from *C. metoecus*, but Nicoll's definition of Stephanophiala is obviously obscure and demands more precision on the one hand and more flexibility on the other.

Redesignation of Stephanophiala. Minute to medium-sized Allocread species with six subequal rounded papillae encircling the oral sucker laterally and dorsally. Prepharynx and esophagus short as in the entire subfamily; pharynx about one-half size of the oral sucker. Forking of the gut a little distance in front of acetabulum. Testes median tandem, larger or smaller than acetabulum. Ovary lateral or median. Uterus composed of a few coils between anterior testis and acetabulum, with the vagina leading directly to the genital aperture. Cirrus pouch a muscular S-shaped sac which may lie entirely dorsal to the acetabulum or may extend a short distance behind, but never extending as far caudad as in Crepidostomum. Genital pore always anterior to the forking of the gut. Vitellaria marginal, somewhat dorsal, from the pharynx to posterior end but usually encroaching on the middle field in the region of the testes and farther caudad. Eggs few to many, averaging about 75μ in length by 45μ in trans-section.

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In this genus are included *Stephanophiala farionis* (O.F.M.) and new species, *S. vitelloba*, from the Bitter Root Valley, Montana.

Stephanophiala farionis (O. F. Müller)

This species was first described by O. F. Müller in 1788. It has passed thru a vicissitude of generic names including Distoma, Distoma (Crossodera), Crossodera, Lobostome, and Crepidostomum. It has more frequently been known as *laureatum* of Zeder than as *farionis*, altho the former specific designation was not applied until some twelve years after Müller's name *farionis* was proposed. In the Old World the species has been described from Central Europe, Scandinavia, and England. In the Western Hemisphere it has been reported from Canada by Stafford (1904) and Cooper (1915) and from Yellowstone National Park by Linton (1893). Several Salmonidae and Percidae have been described as host of this species.

The writer's material for the study of this species in North America consists of parasites of *Salmo mykiss lewisii*, (Gir.) collected by Linton from Yellowstone National Park and described by him (1893) and, in addition, material collected by Dr. B. H. Ransom, from Park Co., Montana, in 1904, and lent by the Bureau of Animal Industry, Washington, D.C. The study of the New World material shows it to belong to the species *S. farionis*, but it shows likewise that the species is widely variable in several characters.

Detailed descriptions of the worm have been given by Olsson (1878: 24), Blanchard (1891:481) and Nicoll (1909:425) and need not be considered here. However, a summary of the constancy and variability of certain characters may well have a place in this paper. In length the mature worm varies from 1.2 mm. (Ransom material) to 6 mm. (maximum record of Olsson). The ventral sucker is always larger than the oral sucker, altho no constant ratio has been found. The fluke has a short prepharynx, a small pharynx and an esophagus shorter than the pharynx. The gut forks a little distance anterior to the acetabulum. The excretory bladder extends to the anterior border of the anterior testis. The testes are median tandem, somewhat larger than the acetabulum. Anterior to them is the ovary, which may be lateral or median in position. Most variable of all is the size and extent of the cirrus pouch. Its posterior limit in some individuals may be near the center of the acetabulum as Nicoll has found in his material, but in other specimens

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it extends behind the posterior margin of the acetabulum. In all cases, however, its internal structure is the same, and the anterior end always extends anterior to the genital pore. The amount of coiling of the uterus is variable. The eggs vary in number from few to about one hundred. They vary in size from 62 to 82μ in length and 40 to 59μ in cross section.

The papillose Allocread species have been uniformly described as aspinose. A small per cent of specimens of the Ransom collection have spines on the integument in the region dorsal and lateral to the oral sucker (fig. 3). They are so definite in character as to leave no room for uncertainty. The small number of individuals of this collection so armed makes it inadvisable to consider them as a distinct variety.

The recognition of the American specimens, especially those of Linton and Ransom as *Stephanophiala farionis* shows the wide distribution of this species. The name *S. transmarina* proposed by Nicoll (1909) for the American specimens of this species consequently becomes untenable and is to be considered as a synonym.

Stephanophiala vitelloba nov. spec.

Stephanophiala vitelloba was taken from the gall bladder of a single specimen of *Coregonus williamsoni* Gir. among several dozen examined from the Bitter Root River, at Fort Missoula, Montana, Feb. 18, 1916. Ten specimens were found at the head of the gall duct.

The worm is quite cylindrical, but tapers in an ovoid fashion at the posterior end. At the anterior end there is a slight bending ventrad, so that the oral sucker is directed anteroventrad. Topping the dorsal portion of the oral sucker are six subequal blunt papillae, about 30 μ wide and 20 μ in depth. About one-third of the distance caudad on the ventral side the acetabulum bulges out as a truncate cone. The fluke is 1.11 to 1.4 mm. long by 0.277 mm. wide at the middle of the body. The oral sucker measures 0.148 mm. in trans-section and the acetabulum, 0.176 to 0.185 mm. The integument is free from spines but has small wartose prominences.

The oral atrium leads thru a very short prepharynx into a barrel-shaped pharynx, 74 μ in diameter and about 100 μ in length. At the posterior end of the pharynx is a short esophagus, behind which the digestive tube bifurcates, each cecum clasping the anterior face of the acetabulum and then running caudad to a position near the posterior

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end of the worm. The wall of each cecum is composed of about eight to ten cells in cross section, with rounded walls lining the lumen of the pouch (fig. 11, *ce*). The cecum is oval in cross section, about 35 μ in frontal diameter and 64 μ along the sagittal plane. The lumen is scarcely as large as the cells of the cecum wall. Each cell is filled with fine granular material and has an oval nucleus 6 μ in section. The general appearance of the cell is vesicular, altho no vacuoles have been found.

The excretory pore is terminal. From it, extending cephalad, near the dorsal face is a long sacculate bladder. It is slightly pouched at the posterior end but soon becomes distinctly tubular. It continues forward as a single tube to the region of the ootype where it bifurcates, sending forward two lateral vessels. Beyond that point the system has not been traced. The bladder has no granular inclusions.

The male genital organs consist of two testes, efferent ducts, muscular cirrus and seminal vesicle. The testes are rounded glands lying one behind the other on the ventral side of the body at the beginning of the posterior half of the body. They measure about 100 μ in diameter. Each testis (fig. 11) contains a great number of spherical cells, arranged almost exactly in concentric rows. The cells are about 6 μ in section. Toward the center of each gland are found various stages of maturation up to spermatids and spermatozoa. The maturing cells occur in large aggregates, so that they form large clumps of densely staining bodies in the gonocoel. From the outer margin of each testis at the anterior end a vas deferens arises. The two efferent ducts proceed separately to the anterior end of the ovary in which region they merge into the posterior end of the cirrus sac. The cirrus is long and muscular. It bends on itself near the middle of the acetabulum and proceeds again forward in the region of the left cecum, ending at the genital pore, anterior to the forking of the ceca. The portion which constitutes the seminal vesicle is located at the S-bend in the sac. Prostate glands occur in the anterior half of the cirrus pouch.

The female genitalia consist of ovary, oviduct, seminal receptacle, Laurer's canal, vitellaria and vitelline ducts, shell gland and uterus. The ovary lies just anterior to the anterior testis, overlapping it in part. It is spherical, lying slightly to the side of the mid-plane (figs. 5, 12, *o*) and measures 110 to 120 μ in diameter. A coil of the uterus lies below it. The immature ovarian cells are similar to those of the testes, but somewhat smaller. The short oviduct proceeds ventrad (fig. 8) where it

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opens into the ootype. On the left of the ootype is a pyriform receptaculum seminalis and branching from its neck is a tubular sac, Laurer's canal, directed dorsad. It ends blindly near the dorsal wall. On the right hand side is a clump of cells, eight or ten in number, with deeply staining cytoplasm and small spherical nuclei. These constitute the shell glands of authors. Extending lateral right and left from the ootype are the vitelline ducts. Their plane practically divides the animal into equal halves. The vitellaria are the most characteristic features of the fluke. Lateral to the ceca they extend from the extreme anterior to the extreme posterior ends. At the posterior end they bend forward inside the ceca, extending almost to the ootype. In section they appear as distinct chorda, the outer series large and at times divided into branches, while the smaller inner glands constitute a single stem. Each chordum in section is composed of aggregates of vesicular cells of irregular polygonal outline and large spherical nuclei which display great numbers of mitotic figures. The entire cell is rich in chromatic materials.

From the ootype the uterus coils forward, opening to the right of the cirrus sac. It contains from four to eight eggs. The eggs are oval, with a lemon colored shell and operculum at one end. They measure about 77 μ in length by 42 μ in trans-section. The egg is filled with granular cytoplasm and has, in addition, from twelve to twenty-four ovate vitelline cells.

Crepidostomum Braun 1900

This genus was created by Braun (1900) to include the species *Distomum farionis* (*laureatum*) and *D. metoecus*, designating papillose Allocreadine species, which unlike Bunodera, have the uterus confined anterior to the anterior testis. The discovery of other species related to these shows that these Old World species are to be regarded as types of distinct genera. The present study shows the close relationship of *Crepidostomum metoecus*, *C. cornutum*, and *C. illinoiense*. On the basis of a study of these three species a redesignation of the genus follows.

Redesignation of Crepidostomum. Minute to inframedium aspinose Allocreadine species with six oral papillae, of which the ventral pair consist of laterally extending muscular processes which taper to an acute point. Dorsal papillae showing a tendency toward bifurcation. Short prepharynx and esophagus; pharynx minute. Gut forking occurs a little or a considerable distance in front of acetabulum, depending on

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expansion or contraction of prepharynx and esophagus. Excretory bladder extending to anterior border of anterior testis. Testes median tandem. Ovary lateral or median, just anterior to testes. Vitellaria from pharynx to posterior end. Vitelline follicles sparsely scattered anterior to ootype. Cirrus sac large, muscular, often convoluted, extending from ootype to front of acetabulum. Genital pore ventral or posterior to forking of gut. Uterus consisting of several coils anterior to anterior testis. Eggs one to several, varying in size from 55 by 40.9 μ for *C. metoecus* to 70 by 41 μ for *C. cornutum*. Found in various fishes in North America and in *Vespertilio lasiopterus* in Europe. Young of American species have been found in Cambarus spp.

Crepidostomum cornutum (Osborn) 1903

This species, originally designated as *Bunodera cornuta*, was described from material from *Micropterus dolomieu*, *Ambloplites rupestris*, and *Ameiurus nebulosus*, taken from Chautauqua Lake, New York. It has since been found in Canada by Stafford (1904:490) in the distomulum stage in Cambarus sp. and by Cooper (1915:193) as adults in *Micropterus dolomieu*, *Ambloplites rupestris*, and *Amieurus lacustris* and in the distomulum stage in Cambarus spp.

The material on which the original description is based (Osborn 1903) bears evidence of being not one species but three species, namely, a Crepidostomum species which must be regarded as the *cornutum* type, *Acrolichanus petalosa* (Lander), and a Bunoderan species, probably *luciopercae*. Specimens from Professor Osborn which the writer has been enabled to examine consist of *Crepidostomum cornutum* and *Acrolichanus petalosa*. The fact that the *cornutum* individuals have a muscular cirrus sac and a short uterus coil never encroaching on the territory of the testes or farther caudad precludes any possibility of regarding them as Bunoderans. While figures 1 to 6 of Osborn's paper are acceptable as representatives of *C. cornutum*, fig. 7 is distinctly a Bunoderan. Thus it becomes necessary to redescribe *C. cornutum* and limit the type more definitely.

The size of *C. cornutum* is variable. Its length ranges from 0.9 to 3.0 mm. and its width from 0.2 to 0.9 mm. While it is slightly narrower at the posterior end than in the region of the acetabulum, the tapering is so gradual as to be almost inconspicuous. The oral sucker is commonly as wide as the body or even wider, its diameter varying from 0.33 to

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0.46 mm. The acetabulum is considerably smaller, from 0.15 to 0.31 mm. in trans-section. It lies in the anterior quarter of the body.

Of the six papillae crowning the oral sucker the ventral pair are lateral extentsions of the sucker itself, giving the appearance on the whole of turned flanges (fig. 14). The four remaining papillae are equal in size and inconspicuous in detail.

The orifice leads thru a short prepharynx into a small oval pharynx about 50μ in cross section. Behind the pharynx is a short esophagus of equal length. The digestive tract usually bifurcates some little distance in front of the acetabulum. Long tubular ceca extend to near the caudal extremity of the body.

The excretory system as far as it has been made out consists of a long bladder arising from a caudal excretory pore and ending near the anterior border of the anterior testis. The tubules have not been studied.

Turning to the genital organs, large testes, capable of considerable elongation or widening, lie tandem in the posterior half of the body. Osborn (1903:69-71) has described slender vasa deferentia which run from the testes along the dorsal side of the fluke, merging into a single duct at the base of the cirrus sac (fig. 6). From ventral aspect the ovary is seen sometimes on the right, sometimes on the left. It is oval and considerably larger than the receptaculum seminalis. The ootype is located in about the center of the body. Its relation to the female genital organs is shown in Osborn's figure 6. The vitellaria are situated in rather definite chorda, closely surrounding the ceca on the sides. Anterior to the ootype they are composed of minute follicles. They usually extend from the pharynx to the posterior end of the body. They do not encroach on the median field as do the follicles in *C. illinoiense*.

The uterus in the mature worm consists of several coils anterior to the anterior testis, with a terminal portion directed forward over the acetabulum toward the genital pore. The genital pore is ventral or slightly posterior to the bifurcation of the gut. Osborn (1903:72) has found its anterior end to be muscular. The eggs of *C. cornutum* are not nunerous; they range up to about twenty. They measure 65 to 71 μ in length by 41 μ in cross diameter. The cirrus sac is a long coiled muscular organ arising in the region of the ootype. At times its convolutions separate the ovary from the receptaculum seminalis. The

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writer has found both the vesicle and the ductus ejaculatorius to be extensively muscular. As is commonly found in the Stephanophialinae the ductus is surrounded by prostate glands.

Crepidostomum illinoiense nov. spec.

This minute fluke was taken from the intestine of the crappie, *Pomoxis sparaides* (Lac.), at Havana, Illinois, July 11, 1910, by Dr. H. J. Van Cleave for Professor Henry B. Ward, to whom the writer is indebted for the material. A large number of specimens were secured from the intestine of the host.

The worm is elongate in outline, with a greatest width of 0.15 to 0.18 mm. in the region of the acetabulum, posterior to which it gradually tapers to a distinctly conical end. The acetabulum measures from 76 to 88μ in trans-section, while the oral sucker is almost twice as large. Crowning the oral sucker is a cluster of six papillae, two ventral, two dorsolateral and two distinctly dorsal (fig. 17). The ventral papillae emerge from the sides of the posterior margin of the oral sucker, but are always intimately connected with an anterior folding of the sucker. Each papilla extends laterad about 15μ then is flexed dorsad some 5 to 7μ, terminating in a distinct point. The dorsolateral papillae are triangular with rounded corners. They extend forward and dorsad. The dorsal papillae lie directly above the orifice. They are separated from one another by an inconspicuous sinus. Each dorsal papilla is bifurcate, altho the notch is not deep. Altho there is no fundamental muscular connection between the dorsal and the dorso-lateral papillae, folds of the integument stretch across the intervening notch, giving the appearance superficially of a sympapillose condition. The body is unarmed.

The mouth opens thru a short prepharynx into a spherical pharynx of 26μ diameter. Behind this organ is an esophagus of equal length. The bifurcation of the digestive tract occurs just a little anterior to the acetabulum. The ceca are long attenuate tubes reaching to the extreme posterior limits of the worm.

The excretory system (fig. 19) was found only in sections. The pore is caudal in position. For a short distance forward the bladder is muscular, but as it bends dorsad its lining is entirely parenchymatous. The tube gradually becomes more and more attenuate in the vicinity of the testes and ends just dorsal to the anterior wall of the anterior testis.

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The ovary is a medium-sized reniform body lying just behind the acetabulum. In ventral view it is covered on the right side by the cirrus pouch and on the left by the uterus. In a median line just behind the ovary lies the ootype, and on the right, posterior to the ootype, is a large pyriform receptaculum seminalis. The testes lie tandem in the third quarter of the body. The anterior one is sometimes compressed longitudinally so that it reaches laterad almost to the ceca. The cirrus pouch is an extremely long muscular organ, originating some distance posterior to the ootype and extending forward over the acetabulum to terminate at the genital pore, ventral to the forking of the gut. It is capable of considerable eversion. The vitelline glands at the margins of the body extend from pharynx to posterior end of the body. Anterior to the acetabulum they are few and small. In the region of the testes they encroach on the median organs and fill the entire dorsal portion of the worm posterior to the testes. The uterus is an uncoiled tube which runs directly to the genital pore. The eggs occur singly or at most in pairs. The mature egg measures 63 by 40 μ. The shell of the immature egg is colorless, while the yolk follicles are yellow; the shell of the mature egg is a dark golden yellow, concealing the color of the yolk material.

Acrolichanus Ward 1918

The name Acrolichanus was substituted by Ward (1918:396) to replace Acrodactyla of Stafford 1904, preoccupied. The genus at the present time includes a single species, first described by Looss (1902:454) as *Distomum petalosum* Lander. The type material was secured from the Lake sturgeon, *Acipenser rubicundus* Le S., from the vicinity of Ann Arbor, Michigan. The species has also been found by Stafford (1904) and Cooper (1915:194, 195) in the same host in Canadian waters. It is highly probable that *Distomum auriculatum* Wedl (?) of Linton (1898: 491) and *Bunodera lintoni* Pratt (Linton 1901:435) are synonyms of *Acrolichanus petalosa*. The type *D. auriculatum* Wedl, described from Europe as a parasite of *Acipenser ruthendus* (Wedl 1857:242, 243) is so inadequately described that it seems unwise to give it a systematic position.

Redesignation of Acrolichanus. Inframedium Stephaliphialine species with six oral papillae, of which the ventral pair is draped over the anterior end of the oral sucker. Excretory bladder dilated with conspicuous constriction at posterior end. Testes median tandem or slightly

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oblique. Ovary close behind acetabulum; vitellaria composed of sparsely scattered follicles. Cirrus sac ending in a large conspicuous sphincter.

Acrolichanus petalosa (Lander) 1902

Acrolichanus petalosa has been referred to frequently in the literature but has never been adequately described. The writer has had access to material and records of this species from the following sources: 1) original drawings of Lander's type material, 2) drawings made by Professor Henry B. Ward of material secured from Cambarus sp., at Ann Arbor, Mich., 1893 (Ward 1894:180), 3) material on which Cooper's record (1915) is based, and 4) specimens of Osborn's material from *Amblopliles rupestris*.

A. petalosa is inframedium in size, averaging from 1.5 to 2.5 mm. in length and 0.32 to 0.54 mm. in width. The acetabulum lies at the posterior limit of the anterior third of the body. It measures 0.16 to 0.32 mm. in cross section. The oral sucker measures from 0.27 to 0.45 mm. The body is aspinose. The disposition of the oral papillae as seen from the front and below is strikingly similar to a notched geranium leaf. The six lobes are practically equal; the ventrals are directed caudad and their folds continue mesad so that they meet in an acute notch just under the oral sucker (fig. 20). A very short prepharynx leads into a pharynx which is at times oval but is more often campanulate. A short esophagus bends dorsad from the pharynx (fig. 21), so that the ceca frequently appear to airse from the base of the pharynx. The ceca originate some little distance anterior to the acetabulum and extend as large pouches to the subcaudal region of the worm. The cells of the ceca are small, from ten to fourteen being found in a cross section.

The excretory bladder arises posteriorly from an inconspicuous caudal pore. For a short distance it consists of a small tube, but soon enlarges into an irregular pouch lined with a thin layer of cells (fig. 26). The pouch ends at the anterior margin of the anterior testis, from which region a pair of lateral tubes can be traced forward for a short distance in preserved specimens. The writer is indebted to Professor Henry B. Ward for Lander's data regarding the details of the tubule system. From the sides of the anterior extremity of the bladder a pair of single tubules arises. Each tubule soon bifurcates, one branch coursing forward and the other caudad. Capillaries ending in flame cells arise along each

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lateral tubule. As many as six flame cells have been found anterior to the forking of the system, while eight have been found posterior to this separation. The anterior limit of the system is in the region of the pharynx.

The ovary, a medium-sized ovoid gland, is usually located lateral to the median line, immediately behind the acetabulum. The receptaculum seminalis is very minute. The testes are large ovate organs, lying in the third or fourth quarter of the body. The ootype is median, just behind the ovary. Laurer's canal which is on the left (fig. 25) meets the duct from the receptaculum seminalis and both open into the ootype from above (fig. 27). The sparsely scattered vitellaria extend from the pharynx to near the posterior end of the body. Their transverse ducts empty into the ootype from a ventrolateral direction.

The uterus emerges from the posterior margin of the ootype. After coiling once it runs forwards to the right of the acetabulum to the genital pore. The eggs are few to several in number, averaging up to twenty-five in some cases. Each egg measures 70 to 72 μ in length by 40 to 50 μ in cross section. The operculum is small but distinct (fig. 23). As in the eggs of the other Stephanophialinae, many yolk cells are found. The small efferent ducts from the testes converge in the plane of the ovary. The muscular cirrus sac begins at this junction. It is daucine in shape, except for a lateral bulge in the portion dorsal to the acetabulum. This bulge is due to the convolution of the seminal vesicle which occupies the middle region of the cirrus sac. The ductus ejaculatorius is conspicuously muscular. Both the seminal vesicle and the ductus are supplied with glands. The opening of the ductus lies posterior to the forking of the gut. It is provided with a powerful sphincter (fig. 27).

The structure of the oral papillae is of a deep-seated character. It is made up of an outermost basement membrane, within which are several enveloping muscle strands, the nuclei of which can be made out plainly (fig. 28). Within the center of the papilla is a core of longitudinal muscle strands. Both of these series are distinct from those making up the oral sucker.

The main features of the worm are made out in very young distomula (fig. 29). These include the campanulate pharynx, distinct genital cells, representing testes, ovary and anterior sphincter of cirrus sac, sparsely scattered vitellaria, and the constriction of the excretory bladder at the posterior end. Eyespots are also present in young worms.

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KEY TO THE SUB-FAMILY STEPHANOPHIALINAE

1 (4) Cirrus pouch small to inframedium, mostly lying over the acetabulum; genital pore anterior to the forking of the gut; papillae subequal . . . . . Stephanophiala 2

2 (3) Oral sucker smaller than acetabulum; testes larger than acetabulum; eggs few to many, varying in size from 62 to 85μ by 40 to 59μ; integument occasionally spinose . . . . . *S. farionis* (O. F. M.)

3 (2) Oral sucker smaller than acetabulum; testes smaller than acetabulum; eggs few, 77μ by 42μ . . . . . *S. vitelloba* Faust

4 (1) Cirrus pouch large, extending some distance behind the posterior limit of the acetabulum; genital pore posterior or ventral to the forking of the gut; ventral papillae large, differentiated 5

5(10) Ventral papillae extending laterad, tapering to acute points; cirrus pouch well developed, muscular, tapering to a small ductus ejaculatorius . . . . Crepidostomum 6

6 (7) Dorsal papillae entire, not tending to bifurcate; pharynx very small . . . . . *C. cornutum* (Osborn)

7 (6) Dorsal papillae tending to bifurcate; pharynx about one-half diameter of oral sucker. 8

8 (9) Inframedium in size, testes very large . . . . . *C. metoecus* Braun

9 (8) Minute, testes relatively small . . . . . *C. illinoiense* Faust

10(5) Ventral papillae extending as folds over the anterior portion of the oral cavity; ductus ejaculatorius with a powerfully muscular end . . . Acrolichanus Single species . . . . . *A. petalosa* (Lander)

Biology of the Stephanophialinae

While no faunistic-biological reconnoissance of the Stephanophialinae will be attempted in this paper on account of the incompleteness of many of the records, the data are nevertheless sufficiently adequate to indicate some of the important biological relations of the group.

With the exception of *Crepidostomum metoecus* Braun all of the described species are parasites of fresh-water fishes. *Stephanophiala farionis* (O.F.M.), the species most widely distributed geographically, has also the greatest host distribution. European investigators have reported it from *Trutta fario* (L.), *T. trutta* (L.), *Epitomynis salvelinus* (L.), *Thymallus thymallus* (L.), and *Coregonus oxyrhinchus* (L.), while American students have found it in *Salvelinus fontinalis* Mitch., *Perca flavescens* (Mitch.), *Eupomotis gibbosus* (L.), *Boleosoma nigrum* (Raf.), *Etheostoma iowae* J. and M., *Stizostedion vitreum* (Mitch.), and *Salmo mykiss lewisii* (Gir.). In addition Stafford (1904:490) has reported this species from *Necturus maculatus*. *Crepidostomum cornutum* (Osborn),

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while limited to the Eastern part of the United States and Canada, has been found in *Micropterus dolomieu* Lac., *Ambloplites rupestris* (Raf.), *Ameiurus lacustris* (Walb.), and *A. nebulosus* (Le S.). *Acrolichanus petalosa* (Lander) has been found in various localities in the Great Lakes and St. Lawrence Basin, in each case from the same species of host, *Acipenser rubicundus* Le S. The species described as new in this paper, *Stephanophiala vitelloba* and *Crepidostomum illinoiense*, have each been found in but a single host in a single locality.

The normal seat of these parasites is the anterior part of the small intestine, altho they have been recorded from the posterior end of the intestine, the pyloric ceca, the gall bladder, and the stomach. It is also a matter of record that the related species *Bunodera luciopercae* (O.F.M.) has wandered out of its host after the death of the latter.

Sufficient data are not at hand to determine annual cycles of infection in the host.

The life history of no one of the species of the Stephanophialinae has been worked out. That there is an intermediate host is a certainty, since immature specimens of *Crepidostomum cornutum* and *Acrolichanus petalosa* have been found in abundance in the crawfish, and cysts of immature *Stephanophiala farionis* have been taken from Hexagenia sp. But the type of cercaria is not known. However, since the cercaria of the near relative, *Allocreadium isoporum*, is a rhopalocercous type and the precocious Allocread species, *Cercaria macrostoma* Faust, is a cystocercous type, it is highly probable that the Stephanophialine species have cercariae that are provided with heavy tails. It is evident that the cercariae have pigmented eyes, since representative species of all three genera of this group show the remains of these eyes in their immature stages. Moreover, since the miracidium of *Bunodera luciopercae* has pigment eye-spots, it is possible that the miracidia of the Stephanophialinae are also pigmented.

A tentative life history scheme for the various species of Stephanophialinae may.be outlined as follows:

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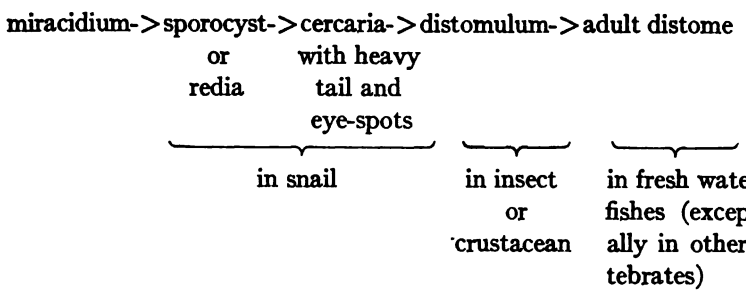

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EXPLANATION OF FIGURES

c.....cirrus pouch
ce.....cecum
d.....vitelline duct
e.....egg
g.....genital pore
l.....Laurer's canal
o.....ovary
p.....pharynx
r.....receptaculum seminalis
s.....shell gland
t_1, t_2.....anterior and posterior testes
u.....uterus
v.....vitellaria
vd.....vas deferens
vs.....seminal vesicle
x.....excretory bladder
y.....ootype

DESCRIPTION OF FIGURES

PLATE XIV

Stephanophiala farionis. 1.—Ventral view, × 54; 2.—lateral view of oral sucker and papillae, × 105; 3.—detail of portion of integument lateral to acetabulum, showing spines, × 180; 4.—egg, × 180.

Stephanophiala vitelloba. 5.—Dorsal view, × 54; 6.—dorsal view of head, showing papillae, × 105; 7.—egg, × 240; 8.—detail of sex organs in region of ootype, × 180; 9.—cross section of worm just anterior to acetabulum, × 180; 10.—cross section thru ovary, × 180; 11.—cross section thru posterior testis, × 180; 12.—cross section thru region of ootype, × 180; 13.—detail of lateral papilla, × 180.

PLATE XV

Crepidostomum cornutum. 14.—Ventral view, × 34; 15.—detail of anterior end, × 24.

Crepidostomum illinoiensis. 16.—Ventral view, × 105; 17.—detail of anterior end, ventral view, × 180; 18.—egg, X 330; 19.—diagram of lateral aspect, × 75.

Acrolichanus petalosa. 20.—Ventral view, × 34; 21.—median sagittal section, × 34; 22.—detail of anterior end, × 24; 23.—egg, × 330; 24.—cross section thru region of genital pore, × 75; 25.—cross section thru region of ootype, × 75; 26.—cross section thru anterior testis, × 75; 27.—detail of genital organs, × 50; 28.—section thru lateral papilla, × 105; 29.—ventral view of young fluke, × 34.

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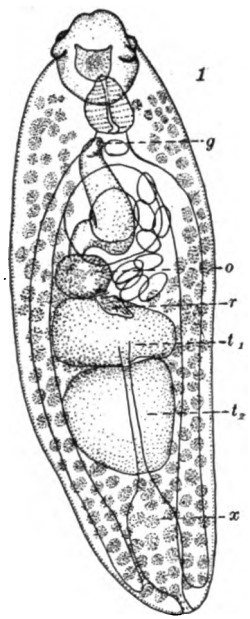

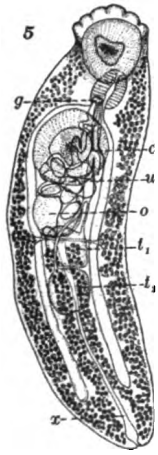

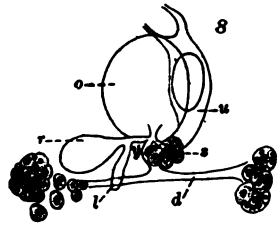

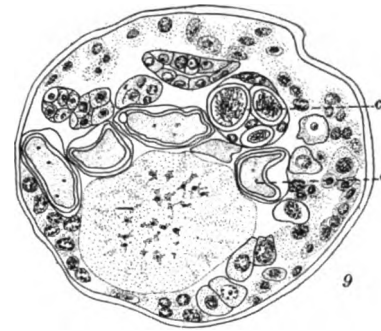

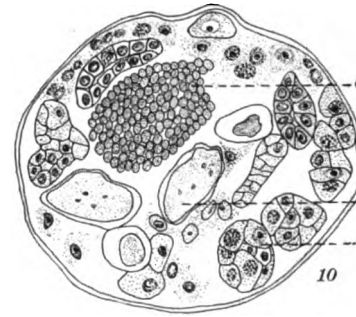

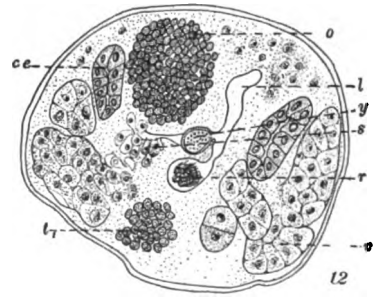

PLATE XIV

FAUST

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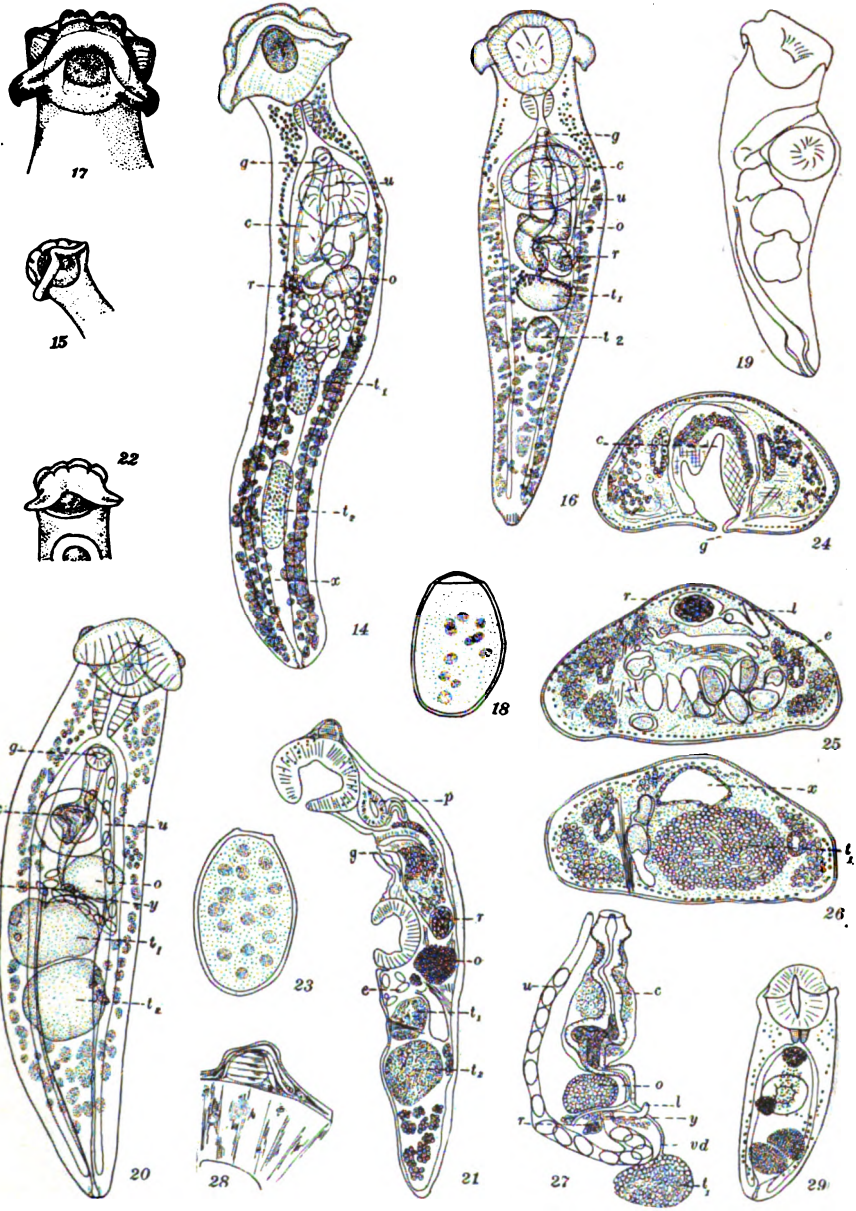

PLATE XV **FAUST**

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DEPARTMENT OF NOTES AND REVIEWS

It is the purpose, in this department, to present from time to time brief original notes, both of methods of work and of results, by members of the Society. All members are invited to submit such items. In addition to these there will be given a few brief abstracts of recent work of more general interest to students and teachers. There will be no attempt to make these abstracts exhaustive. They will illustrate progress without attempting to define it, and will thus give to the teacher current illustrations, and to the isolated student suggestions of suitable fields of investigation.—[Editor.]

AQUATIC MICROSCOPY

To those of us who remember the first thrills of work with the microscope it is not easy to understand why there are not more amateur students of nature who cultivate a life interest in the microscope. Very much of the early enthusiasm of this Society was given to it by those who were using the microscope in amateur exploration of the marvels of nature. The American schools and colleges make much use of the microscope, but those who are most interested in these things tend to go on into the study of some more or less technical aspect of the subject. It would seem however, if our teaching were as effective and inspiring as it should be, that more of our students would thruout life continue an amateur interest in this charming field.

Perhaps, as we grow older in America and achieve more leisure and more general interest in culture, we may again increase the number of those who untechnically use the microscope for the mere love of its interesting revelations. In England there are many such, and numerous simple handbooks have been issued which helpfully guide the beginner. Dr. Stokes has been a long-time exponent and writer for such students in this country. His little book, now in its fourth edition, has done its part to keep alive amateur interest. The author undertakes in the simplest and most concrete way possible to answer the questions of the embryo naturalist, such as:—"How can I best find and collect the microscopic plants and animals? How can I tell them apart and determine what they are and how they are named? What outfit will I need, and how can I prepare my material for profitable study?"

These questions are answered in the following chapters: I, The Microscope and its Parts; II, Common Aquatic Plants Useful to the

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Microscopist; III, Desmids, Diatoms, and Fresh Water Algæ; IV, Rhizopods; V, Infusoria; VI, Hydra; VII, Aquatic Worms (and Insect Larvæ); VIII, Rotifera; IX, Freshwater Polyzoa; X, Entomostraca and Phyllopoda; XI, Water Mites and Water Bear.

The especial virtues of the book are in its untechnical language, its simple descriptions, its concrete keys and synopses, and its outline drawings. The author states that, with the exception of a few western forms, every type of organism mentioned in the book was taken by him from a single pond in central New Jersey. Aside therefore from its value as a guide for the study of nature, it has a value as a contribution to intensive study of a limited locality. The same features which make it useful to the independent beginner will make the book helpful to the students of elementary Biology in high school and college, and stimulate to more effective field work.

AQUATIC MICROSCOPY FOR BEGINNERS, by Alfred G. Stokes. Fourth edition, revised and enlarged; 324 pages, illustrated. John Wiley & Sons, New York and London. Price $2.25 net, postpaid.

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Albert McCalla, M.A., Ph.D., F.R.M.S., died suddenly of heart failure at 9:15 P.M., on Thursday, June 6th, 1918, at his late residence, 2316 Calumet Avenue, Chicago. He was 72 years of age and his demise came after more than a year of ill health. He was the son of Thomas McCalla, one of the first bankers of Chicago, and Marianne Davisson, and was the brother of the late Mary Ella McCalla. Of Scotch descent, he was of South Carolina and Virginia lineage.

Mr. McCalla was much interested in scientific research and received a number of degrees. He was possessed of unusual expert ability with the microscope and was the inventor of an attachment widely used in days gone by.

He graduated from the old Chicago High School and was the winner of the Foster Medal; graduated from Monmouth College; was one of the founders of Beta Theta Pi at the old Chicago University. He was a member of the following organizations:

Fellow Royal Microscopical Society, London.
Past-President American Microscopical Society.
Past-President Illinois Microscopical Society.
Member American Association for the Advancement of Science.
Member First Presbyterian Church, Chicago.
Member Sons of the Revolution.
Member Beta Theta Pi Fraternity.

He taught at Parsons College and later at Lake Forest College.

He is survived by his wife, Eleanor Hamill McCalla, daughter of the late Honorable and Mrs. Smith Hamill, and four children, Helen Wayne McCalla, Thomas Clarendon McCalla, Major Lee A. McCalla, U. S. A., and Paul Hamill McCalla.

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OCTOBER
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VOLUME XXXVII NO. 4

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Microscopic work

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2. Resulting discoveries must first be offered for publication in the Society Transactions.
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NOTICE TO MEMBERS

It is a source of regret to the Editor that the *Transactions* cannot be issued as dated. The issue is dependent, however, upon the income of material from the members, and must await upon a certain degree of balance in the material as well as quantity of material.

The Secretary will consider it a favor if members will notify him of non-receipt of numbers of the Transactions, and of changes of address.

Correspondence relative to membership, publication, exchanges, advertising, etc., should be addressed to the Secretary-Editor.

T. W. GALLOWAY,
Secretary-Editor.

Beloit, Wisconsin.

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TRANSACTIONS
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A NEW SPECIES OF RHYNCHELMIS IN NORTH AMERICA*

F. Smith and L. B. Dickey

The worms described in this paper are part of a series of Oligochaeta obtained by Miss Bessie R. Green from the vicinity of Flathead Lake in Montana during the summer of 1914, while at the Biological Station maintained by the University of Montana. The Rhynchelmis specimens were collected in July by A. G. Vestal and M. J. Elrod, for whom the species is named, from a creek near the Station, and included several mature specimens and a number of immature ones.

But two species and a variety of Rhynchelmis have previously been known. *R. limosella* Hoffmeister is a common European species, and the Asiatic species *R. brachycephala* and its variety *bythia* have been somewhat recently made known by Michaelsen (1901 and 1905). We now describe a distinct but somewhat closely related species from North America.

A modification of the definition of the genus in a few characters is necessary, and a still closer relationship between Rhynchelmis and the North American genera Sutroa and Eclipidrilus becomes apparent.

Rhynchelmis Hoffmeister

Setae simple. Spermiducal pores paired on 10.† Oviducal pores paired in intersegmental groove 11/12. Spermathecal pores paired on 8. Longitudinal muscle layer completely separated into eight longitudinal bands. Transverse blood vessels, two pairs, in each of most somites. Spermaries and spermiducal funnels paired in 10, or two pairs in 9 and 10; sperm ducts, one or two pair, opening into a pair of long atria. Ovaries paired, in 11. Spermathecae paired, in 8, without diverticula opening into the spermathecal ducts, ampullae communicating with the alimentary tract.

* Contributions from the Zoological Laboratory of the University of Illinois, No. 106.

† Arabic numerals are used to designate the somites, counted from the anterior end.

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RHYNCHELMIS ELRODI SP. NOV.

Length, 47-65 mm. Somites, 133-177. Proboscis long and slender. Setae closely paired. Clitellum on 9-17. Spermiducal, oviducal, and spermathecal pores nearly in seta line *ab*. Longitudinal muscle bands not spirally rolled at edges. Ventral vessel forked in 7, and connected with dorsal vessel in 1. First nephridia in 13. Spermaries paired, in 10. Spermiducal funnels, one pair; sperm ducts, one pair, imbedded in the walls of the atria. Albumen glands lacking. Spermathecae, one pair in 8; communicating by ducts with the alimentary tract.

From the mucky banks of a creek near the Biological Station at Flathead Lake in western Montana.

Holotype and paratypes in the collection of the senior author (Cat. No. 1058).

The more important facts of structure were gained from the study of a series of sagittal sections of the 33 anterior somites of one specimen, and of two series of transverse sections from the anterior 18 somites of each of two other specimens, of which one is the type.

EXTERNAL CHARACTERS

Alcoholic specimens, apparently sexually mature, are 47-65 mm. in length, and 0.9-1.25 mm. in diameter in the region of the clitellum, where the diameter is greatest. In the anterior half of the worm the body is nearly circular in cross section, unlike other described species of Rhynchelmis, and elsewhere it is not decidedly quadrilateral. In one apparently complete specimen, the number of somites is but 133, while in another it is 177. The number of somites in other specimens varies between these extremes and approximates 150. The anterior part of the prostomium is prolonged into a slender tentacle-like proboscis. The setae are closely paired and the distances between the pairs are approximately indicated by the formula; *aa**:*bc*:*dd* = 3:5:5. The setae are sigmoid, slightly more curved at the distal end, slender, and simple. The average length is about 0.27 mm., and the diameter at the nodulus is about 0.01 mm. The nodulus is at about one-third of the length of the seta from the distal end.

The clitellum is developed on 9-17 and encroaches slightly on the adjacent somites. It is most strongly thickened on 10-16, and is devel-

* Letters are used to designate the setae of either side of a somite, beginning with *a* for the most ventral one and proceeding in order to *d* for the most dorsal one.

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oped ventrally as well as dorsally. The spermiducal pores are paired on 10, slightly anterior to 10/11, and nearly in line with the ventral setae, The oviducal pores are small, in 11/12, and in line with the ventral setae. The spermathecal pores are paired on 8, posterior to the ventral setae.

Internal Characters

The brain lies dorsad of the mouth, in the first somite, and is similar in form to that of *R. limosella*, as figured by Vejdovský (1876). The ventral nerve cord is closely adherent to the body wall throughout its length. The layer of longitudinal muscle fibers is in eight distinct bands, as in other species of the genus, but the edges of these bands are not rolled as in *R. limosella* (Vejdovský, 1884, pl. 16, figs. 1 and 2), and in *R. brachycephala* and its variety, as described by Michaelsen (1905:62–63). The alimentary tract is simple in character, like that of the other species.

The ventral vessel forks in 7 and the two anterior branches unite near the brain with the dorsal vessel. A pair of transverse vessels in the posterior part of each of somites 2–6, connect the dorsal vessel with the branches of the ventral; and similar transverse vessels in 7–12, connect the dorsal and ventral vessels. In one specimen there is a similar vessel on one side of 13. The paired posterior transverse vessels of somites posterior to 12 are connected with the dorsal vessel only. They have a few caecal branches and often extend only part way down the sides of the body. There is a pair of transverse vessels in the anterior part of each of most somites posterior to 7. The first pair are somewhat shorter and more simple, but those of somites posterior to 8 extend to the ventral side and have several caecal branches. In the somites that have been examined, posterior to 12, each of these vessels is connected with the ventro-lateral wall of the intestine by a branch which extends obliquely dorsad and mesad from that part of the vessel lying in the ventro-lateral part of the body cavity. Ventro-intestinal vessels connect the ventral vessel with the ventro-median wall of the intestine (fig. 1). In somites 10 or 11 to 18 or 19 inclusive, these vessels, usually three in number, enter peculiar glandular bodies which are closely associated with the ventro-median wall of the intestine and correspond to the blutdrüsen described by Michaelsen (1901:178) in *R. brachycephala*. These blood glands (fig. 1) are more intimately united with the wall of the intestine in *R. elrodi* than are those of the other species.

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In the specimens examined, the most anterior nephridia are in 13 or 14, and they are more or less irregularly distributed posteriorly. There are sometimes a pair in a somite, sometimes a single one, and often none at all. Just posterior to the septum which supports the nephridial funnel, there is an enlargement similar to that found in a considerable number of other species of lumbriculids. The nephridiopores are in the line of the ventral seta bundles and a short distance anterior to them.

There is but one pair of spermaries and they project freely into 10 from their attachment to the posterior face of 9/10. A pair of sperm sacs extend posteriorly on either side of the alimentary tract, from their openings in septum 10/11, at least as far as to somite 30, in some specimens. The spermiducal organs are similar in their main features to those of other species of the genus; but there is no trace of more than one pair of sperm ducts or spermiducal funnels, and those present belong to somite 10. The funnels are on 10/11, below and laterad of the openings of the sperm sacs, and the dorsal edges of the funnels extend into the sacs, along their ventral wall for a short distance. In tracing each sperm duct from the funnel towards the external pore, we have a relatively slender duct which extends posteriorly through several somites in the cavity of the corresponding sperm sac, to a position at which it enters the posterior end of a much larger and tubular atrium which extends anteriorly into 10 and then, bending ventrally, joins the body wall, posterior to the ventral setae, and opens to the exterior at the spermiducal pore. There is a general correspondence between the main features of the spermiducal organs, as outlined above, and those of the other species of the genus; but a more detailed study yields distinct differences, as will appear later. From the funnel the sperm duct first extends ventrad along the septum and then anteriad to the atrium which it follows closely to the place of their union. The duct and atrium are merely in contact in somite 10, but in the anterior part of the sperm sac the duct becomes more strongly flattened against the atrial wall, and about opposite 11/12, in the type specimen, it enters the tissue of the atrial wall (fig. 2, *sd*) and follows it to a point near the posterior end of the atrium, where duct and atrium merge and their cavities become continuous. This intimate relation of duct and atrium is more like the condition found in certain species of Eclipidrilus than it is like that of the other species of Rhynchelmis. In the type specimen the atrium extends posteriorly to 15, and in the other sectioned specimens not so far. Numerous small glandular masses

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or prostate glands which are much like those of other species of the genus, are attached to the outer surface of the atrium (fig. 2, *pr*). The ectal ends of the atria are apparently protrusible and may function as penial organs. In somite 9, in other species of Rhynchelmis, there are organs, either one or a pair, which are known by various names: albumen glands, Kopulationsdrüsen, etc. There are no recognizable traces of such organs in *R. elrodi*.

There is but one pair of ovaries and these are in 11 and are attached to the septum 10/11. The paired ovisacs extend posteriorly from 11/12 and closely invest the corresponding sperm sacs except where ova prevent. They extend through several somites posteriad of the sperm sacs. Paired oviducal funnels are on the anterior face of the septum 11/12, and the very short oviducts open to the exterior in the segmental groove 11/12 in line with the ventral setae. Paired spermathecae in 8, correspond closely with those of the other species of the genus. They open to the exterior posteriad of the ventral seta bundles of 8; the ducts are without diverticula; and the ampullae open through narrowed duct-like portions into the alimentary tract. In one specimen the spermathecae have no connection with the alimentary tract and the diameter of the lumen is much less than normal. This is probably due to degeneration, since the spermaries are small and apparently at a stage of inactivity and yet the sperm sacs are well distended with sperm cells.

Systematic Relationships

The new species has important characters that ally it closely with the Eurasian species of Rhynchelmis, and others in which it is nearly related to Sutroa (Beddard, 1892; Eisen, 1888, 1891) and Eclipidrilus. The simple pointed setae, and much elongated atria are characters shared by all of them. In having an intervening somite between the spermathecal and atrial somites; in the communication between the spermathecae and the alimentary tract; and in the lack of differentiation of each atrium into a "sperm reservoir" or "storage chamber" and a penial organ with narrowed connecting duct; it resembles the species of Rhynchelmis and Sutroa and differs from those of Eclipidrilus (Michaelsen, 1901:150; Smith, 1900:473). It is nearer to Rhynchelmis than to Sutroa, in having the spermathecae paired and without diverticula; but resembles the latter rather than the previously known species of the former, in having no atrial remnants (albumen glands) in somite 9. To the writers the rela-

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tionships to Rhynchelmis seem more significant and they include it in that genus. One important difference between Rhynchelmis and Sutroa disappears when we find simple, paired spermathecae, and absence of atrial remnants in 9, characterizing the same species. It is interesting to note that the possibility of the existence of such a species of Rhynchelmis as *R. elrodi* has already been forecast by Michaelsen (1908:163).

"Ich bin in meinen Betrachtungen dieser Reduktionsverhältnisse dann noch einen Schritt weiter gegangen. Von *Rhynchelmis brachycephala* ausgehend, sagte ich mir, dass es kein morphologisch sehr bedeutsamer Vorgang sei, wenn nun die rudimentären, Samentrichterlosen Samenleiter des vorderen Paares und die verlassenen, ihrer Hauptfunktion enthobenen Atrien des vorderen Paares ganz schwänden. Es würde dann ein Zustand des männlichen Geschlechtsapparates eintreten, der mit dem ursprünglich einfachpaarigen Apparat durchaus übereinstimmte."

In *R. limosella* (fig. 3) there are two pair of spermaries and spermiducal funnels in 9 and 10, and two pair of sperm ducts joining the paired atria of 10. In 9 there are paired organs resembling atria but without the atrial function since no sperm ducts are connected with them. They are the "albumen glands" and presumably represent an additional pair of atria which in ancestors were joined by the sperm ducts connected with the spermiducal funnels of 9. In *R. brachycephala* (fig. 4) and its variety *bythia*, the spermaries and spermiducal funnels of 9 have disappeared and there is a partial disappearance of the related pair of sperm ducts, while the atrial organs of 9 are still represented. In *R. elrodi* (fig. 5) there is a complete disappearance of the reproductive organs of 9, and we have simply the single pairs of spermaries, spermiducal funnels, sperm ducts, and atria in 10. We also have a single pair of ovaries and of oviducts which are in 11. The location of the single pair of spermathecae in *R. elrodi*, two somites anterior to the one containing the male organs, which would otherwise seem rather peculiar, is easily understood on the assumption that this species has been derived from ancestors similar to *R. limosella*. In accordance with the views of Michaelsen, these in turn were presumably derived from Lamprodrilus-like ancestors in which each pair of sperm ducts had its own pair of atria independent of others.

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EXPLANATION OF PLATE XVI

Fig. 1. *Rhynchelmis elrodi.* Transverse section through the posterior part of somite 17: int, intestine; bg. blood gland; vi, ventro-intestinal vessel; vv, ventral vessel; ss, sperm sacs.

Fig. 2. The same. Transverse section through the atrium near the place of entrance of the sperm duct: at, atrium; sd, sperm duct; pr, prostate glands; s, developing sperm cells. Semi-diagrammatic.

Fig. 3. *Rhynchelmis limosella.* Diagram showing relations of the reproductive organs of one side: sy, spermary; sd, sperm duct; at, atrium; al, albumen gland; oy, ovary; od, oviduct; st, spermatheca.

Fig. 4. *Rhynchelmis brachycephala.* Similar diagram: adapted from figure of Michaelsen (1901:179).

Fig. 5. *Rhynchelmis elrodi.* Similar diagram.

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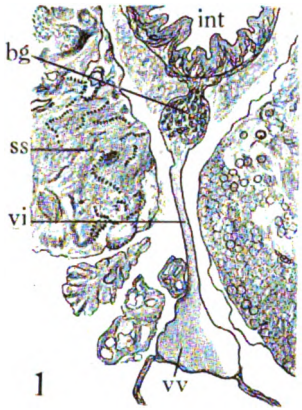

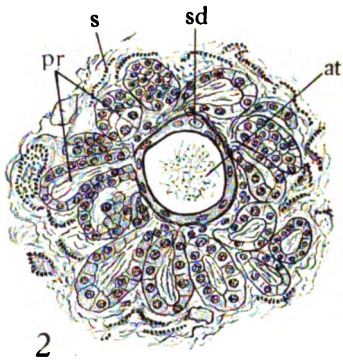

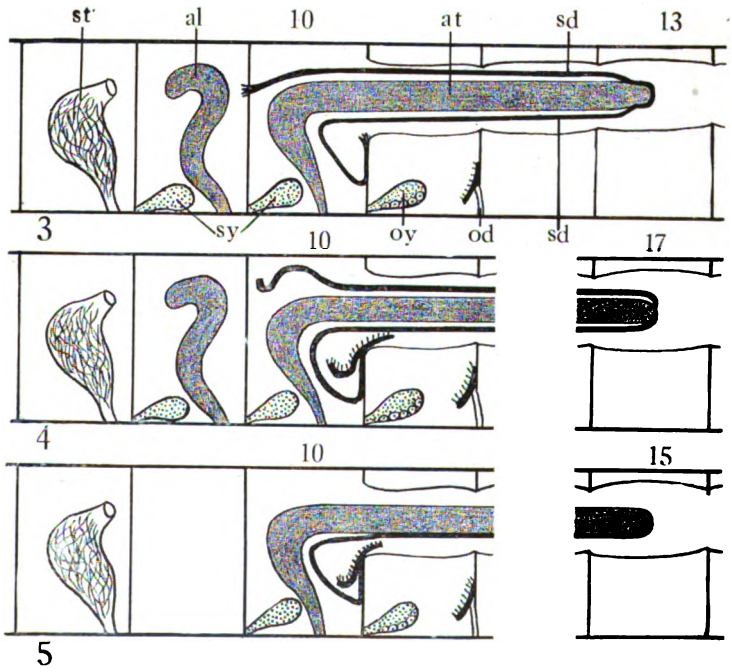

PLATE XVI **SMITH AND DICKEY**

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DEVELOPMENT OF THE WOLFFIAN BODY IN SUS SCROFA DOMESTICUS

EDWARD J. ANGLE, A.M.M.D.

The results embodied in this paper are from studies undertaken some years ago. At that time it was realized that the investigation was far from complete and publication was delayed with the hope of further study—a hope never realized—of the source of origin of the several portions of the urinary tubules. In the light of recent research the publication of the paper at this late date is largely that the illustrations may prove of some permanent value.

HISTORICAL

The Wolffian bodies (Corps de Wolff, Urniere, Mesonephros, Primitive Kidney) were discovered by Casper Fr. Wolff in the year 1759, who regarded them as representing the embryonic period of the true kidney (Metanephros). They received their present name from H. Rathke in 1825; this only applied to the Wolffian bodies of birds, as Rathke termed the same organs in mammals Okensche Körper. In 1824 Jacobson introduced the name of primordialniere and discovered that in birds these bodies secreted uric acid, which was conducted away by the allantois. The first mention of the mesonephros in man was made by J. Frey Meckel (1809) in his work on comparative anatomy. Meckel describes in fairly accurate language the mesonephros of an embryo 1 mm. long; but evidently was in doubt as to what the organ was, as he later asks the question—"are these structures the common source from which lungs, liver, kidneys, adrenals and sexual organs later have their origin?"

The older writers (Wrisberg, Dzondi, Oken, Emmert and Hochstetter) had many fanciful theories regarding the role of the Wolffian body, regarding it either as the beginning of the kidney or as the horn of the utereus.

Rathke (25) led the way to a true conception by discovering the origin of the true kidney in an embryo chick of six days and showed that the Wolffian body was related to the kidneys as the gills are to the lungs.

The glomerulus of the mesonephros was discovered by J. Müller (30). The honor is due Müller for having first accurately and correctly followed the developmental changes of this organ in a large series of embryos.

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The smallest of Müller's embryos had a length of 20 mm. In this embryo he describes the adrenals, which are quite large and covered by the true kidney, the kidney and ureter, the Wolffian bodies with their conducting sexual portions. The Wolffian body is described as a long flat organ which is in relation on its lateral surface with the sexual duct. Müller emphasizes the fact of the early disappearance of the Wolffian body in man; for in his next embryo which was 3 cm. long he found between excretory duct and sexual gland (does not mention whether testes or ovary) a long spur which is the remaining trace of the Wolffian body. The chief merit of Müller's work consists in his having discovered the rôle which the Wolffian body plays in the development of the sexual ducts.

Among the noted early investigators was Valentin (35) whose work principally relates to mammals. In the Wolffian body, Valentin distinguished two portions; an outer half which consists only of canals and an inner portion made up principally of coiled vessles (Malpighian bodies). Valentin adds parenthetically that in spite of great pains it is frequently difficult to determine the direct connection of the Wolffian tubules with the Wolffian duct.

When one considers the imperfection of optical instruments in use at the period when Müller, Rathke and Valentin lived, one can only wonder at the accuracy of their observations. In mammals, von Baer (37) says, that the primordialnieren arise and disappear as in birds and that their structure clearly points to the general characters of secretory glands.

Bischoff (42) remarks that the Wolffian bodies are only to be found in very young human embryos and that in the second month only faint traces of the glands are to be found. This author agrees with J. Müller, Rathke, Jacobson, E. von Baer that the Wolffian body is an excretory organ. Koebelt (47) observed the atrophy of the Wolffian body in man and higher mammals and from his work concludes that the epididymis of the male is a homologue of the epoophoron in the female.

Waldeyer (70) devoted his attention to the early developmental changes of the Wolffian body in the chick, mammals and man and found the phases of development in the two latter in no way different from the former. From the fact that the Wolffian canals in their several portions are lined with different forms of epithelium, Waldeyer came to the conclusion that there were two types of canals in the gland and from this fact differentiated them into a sexual and a urinary portion; from the former arises the epoophoron or epididymis and from the latter the

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paroophoron or paradidymis. This opinion was concurred in by Rathke, Dursy and J. Müller.

All investigators prior to 1870 regarded the Wolffian tubules as arising either as evaginated blind sacks from the Wolffian duct or from differentiation of the mesoblastic tissue of the middle plate.

In the year 1874 a new theory was promulgated by the independent investigations of three men, Semper (75), Balfour (74) and Schultz (75) who came to the conclusion that in Selechians the segmental Wolffian canals are in relation with the coelom by means of nephrostomes. According to Semper these segmental canals arise from hollow invaginations of the pleuro-peritoneal epithelium. Balfour regarded the canals as arising from solid buds from the intermediate cell mass, the buds later acquiring a lumen.

Götte (75), working independently of the preceding authors, found in amphibia that the Wolffian tubules arise as hollow outgrowths from the urogenital fold of the peritoneum. This observation was confirmed by Spengel (76) and Fürbringer (78). Spengel and F. Meyer, working independently discovered in 1875 that the amphibian Wolffian body possesses peritoneal funnels and the former regards the Wolffian body of amphibians as possessing a segmental formation and holds that its peritoneal funnels deserve as in Selachians, the name of segmental nephrostomes. Fürbringer (78) has shown in Petromyzon that the first anlage of the Wolffian body originates from segmentally arranged cell cords which arise from the peritoneal epithelium.

In 1875 Kölliker (75) investigated the origin of the Wolffian tubules of amniota which agrees with the anamnia in its essential features. In reptiles according to Braun (77) the Wolffian tubules arise from buds which had previously been evaginated from the coelom epithelium; these buds are segmental as in Selachians and solid as in mammals, and become segmental vesicles. The connection with the peritoneum soon vanishes. Thus we see in the seventies a strange concord of opinion; in all vertebrates which had been investigated the theory of Semper and Balfour regarding Selachians was confirmed.

Sedgwick (80) advanced the view later that the Wolffian tubules in the chick do not arise as peritoneal evaginations as announced by Semper and Balfour but arise through a differentiation of the Wolffian Mesoblast. Soon after this Weldon (83) showed for Lacerta that the Wolffian tubules do not originate, as held by Braun, from peritoneal evagination but

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according to the theory of Sedgwick. The theory advanced by Sedgwick for birds and Weldon for reptiles was opposed by Janosik (85) who had investigated the subject in the chick. Mihalkovics (85) agrees with Sedgwick and Weldon that in Sauropsida the Wolffian canals do not arise as a growth from the coelomic epithelium but by differentiation of the Wolffian mesoblast.

Hoffmann (89) investigated the Wolffian canals of Lacerta and found that they arise similar to those in Selachians, with the difference that the constriction of the nephrostome from the lateral plates occurs at an earlier period.

According to Martin (88) in rabbits the anlage of the canals is differentiated from the middle plate and later loses connection with the mesoblastic somites.

Kollman (92) is authority for the statement that the middle plates in amniota are segmental. This conclusion was arrived at by surface observation and confirmed by sections. In man the same conditions could not be directly shown but can be assumed as the Wolffian canals are arranged segmentally and the Wolffian vesicles show segmental characters.

In the same year Field (91) made a study of amphibians. He came to no conclusions in Amblystoma although he considers it probable that the tubules arise from a proliferation of the peritoneal epithelium but not from a true invagination.

Description

The Wolffian body is the chief occupant of the embryonic Wolffian ridge; in Anamnia it is the chief renal organ throughout life; in Amniota on the contrary it disappears during embryonic life, being entirely replaced by the true kidney (metanephros), with the exception of a small portion of the cephalic end which is retained and becomes a constituent of the developing sexual gland.

In its primitive form the Wolffian body consists of a series of transverse tubules emptying into the Wolffian duct. As Sempter (75) has shown for Plagiostomes there is one tubule for each body somite.

A mesonephros in the simple form in which it is first produced developmentally is retained permanently, as Gegenbaur has shown, only in Bdellostoma, a species of Cyclostomes. Here the organ consists according to J. Müller of short transverse tubules whose proximal closed ends

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are invaginated by glomeruli and which open after a short course into the Wolffian duct. In all remaining vertebrates the mesonephros is metamorphosed into a more voluminous and complicated organ and shown manifold changes over the simple form. Here we find a distal strongly convoluted tube opening into the Wolffian duct by means of a collecting tube; the proximal distended portion of the canal becoming a Bowman's capsule and lastly a peritoneal opening leading up to the glomerulus. This latter however is not found in the amniota as maintained by Hertwig (92) who claims that it is present in the three higher classes of vertebrates. I have searched diligently for traces of this in the chick, rabbit, cat and pig, and have found no evidence of the presence of such a canal. The Wolffian body develops in the intermediate cell mass which is formed when the mesoblastic somites are constricted off from the lateral plates; it arises through a union of the median portions of the latter and is best known under the name of middle plate. It has been amply shown that the coelomic epithelium of the middle plate represents without exception the anlage of the sexual glands and I shall attempt to show that the middle plate itself represents the anlage of the excretory apparatus and that the latter contains no traces of coelomic epithelium; thus is shown the descent of the entire anlage from mesoblast.

Preceding the appearance of the anlage of the Wolffian tubules there appears an accumulation of mesoblastic cells on the mesial and ventral side of the Wolffian duct. These cells assume a radial appearance and become hollowed out to form small vesicles. These vesicles were termed mesonephric vesicles by Remak (50) and segmental vesicles by M. Braun (77). Braun found in lizards the number of vesicles to correspond with the number of segments but in birds Mihalkovics (85) has found the vesicles more numerous than the mesoblastic somites. In Sus I have found from two to three vesicles for each somite and consequently the term segmental vesicle of Braun is inappropriate for birds and mammals. The Wolffian vesicles are either oval or circular in outline when viewed in sections and are lined with columnar epithelium. These epithelial cells have large clear and well defined nuclei and each cell possesses a deeply staining nucleolus. In Figures 1, 2, and 3, the relations of the Wolffian vesicles to surrounding parts are clearly shown; Figures 1 and 2 are transverse sections from the proximal portion of the Wolffian body of an embryo 2.5 mm. long. The Wolffian vesicle (w. v.) is seen in the above figures to be situated ventral and medianwards from

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the Wolffian duct (w. d.) and is in close relation ventrally with the coelom epithelium of the middle plate. Dorsally the vesicles are in relation with the mesoblastic somites (m. s.) and medianwards with the aorta (a). The Wolffian duct in these figures has not as yet acquired a lumen. Just posterior and a trifle lateral to the Wolffian duct a small blood vessel is visible, this is the vena cardinalis (v. c.) which is closely related to the growth of the Wolffian body. As the Wolffian body grows and enlarges the cardinal vein is forced to assume a position dorsal to the Wolffian ridge. Its position is readily seen in figures 7, 21, 9 and 10. The first two of these sections are from embryos three mm. long and the two latter from embryos four mm. long. Its shape varies greatly as will be seen in comparing the figures 9 and 10. In embryos a little older (5 mm.) it will be seen in figures 12, 18 and 19 that the cardinal vein (v. c.) is situated near the dorso-median angle of the Wolffian ridge, and is in close relation with the Malpighian bodies (m. b.) which are fully developed in embryos of 5 mm. length.

The Wolffian vesicles (w. v.) are shown in an oblique section in figure 3, which is from the distal end of the Wolffian body of a three mm. embryo. The vesicles here are in relation laterally with the Wolffian duct (w. d.) and medianwards with the aorta (a). The small amount of mesoblastic tissue surrounding the vesicles is particularly noticeable.

The origin of the anlage of the Wolffian canals is a subject which has engaged the serious attention of embryologists for the past score of years and has given rise to a voluminous literature. Among the amniota, birds have received the attention of a majority of investigator, reptiles still less and mammals least of all, which is not at all commensurate with their position and importance in the animal scale. The names of Kölliker, Renson, Kollmannm, Egli, His, H. Meyer, Nagel and Mihalkovics are in the foreground of investigators on the development of urogenital organs of mammals.

Three views have been advanced for the origin of the Wolffian tubles:

(a) *The Wolffian Tubules Arise Similarly to the Tubules of Other Glands That is as Hollow Evaginations from the Wolffian Duct.* This theory was advanced by Remak in 1850 and was accepted by Waldeyer (70) with the distinction that the tubule only is an outgrowth from the duct, the Malpighian body arising from the mesoblast independently and later joins the tubule. It is only necessary to examine sections of young

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embryos in which the Wolffian vesicles are yet separate from the Wolffian duct to show the incorrectness of this view (w. v., Figures 1, 2 and 3).

(b) *A More Modern Theory is that the Tubules take their Origin from an Evagination of Cell Cords or Buds from the Coelomic Epithelium of the Middle Plate.* This was first advanced by Balfour (75) and Semper (75) for Selachinas. Among workers on amniota the adherents of this theory are Braun (77), Weldon (83), Kölliker (79), Kollmann (82), Siemerling (82), Sedgwick (81) and Rensen (83). Sedgwick held this theory only for that portion of the Wolffian body which develops anterior to the sixteenth mesoblastic somite. In reptiles according to Braun the tubules arise from funnel shaped invaginations of the coelom epithelium which are then constricted off from the latter and become the segmental vesicles, and which are present in numbers corresponding to the body segments. These vesicles secondarily unite with the Wolffian duct. The vesicle proper becomes the future Malpighian body while the tubule arises from a short canal which connects the vesicle with the Wolffian duct. Weldon holds the same theory as Braun but merely makes the statement without any evidence.

According to Kölliker in embryo chicks of the sehond day, there are to be seen, on the median side of the urogenital ridge club-shaped buds of epithelial cells which are growing in towards the connective tissue of the Wolffian blastem. Kölliker observed fine fissures in these cell cords, which he regarded as portions of the coelomic cavity constricted off with the cells. The connection with the coelomic cavity is lost only after the tubules have made their union with the Wolffian duct. Kölliker observed the same in the rabbit with the exception that no fissures were present.

Kollmann examined embryos of mouse and rabbit and confirmed in toto the view of Kölliker. Renson (in chick, rabbit and rat) divides the Wolffian body into two portions, a proximal extending from the seventh to the eleventh somite and a distal, extending from the eleventh somite to the pelvis. In the first named region the tubules arise from isolated buds of the pleuro-peritoneal epithelium while in the latter the tubules are differentiated from the intermediate cell mass which had previously arisen from an ingrowth of the pleuro-peritoneal epithelium in the form of a longitudinal plate. The cells which become anlagen of canals are arranged around small lacunae. The lacunae are the remains of small

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fissures when the longitudinal plate was constricted off from the coelom epithelium. The remainder of the lacunae form the cavities of the Malpighian bodies. Renson regards the pronephros and mesonephros as being homologous organs; a view which is untenable at the present time.

Hertwig (92) in his text book of embryology says: "The collective evidence of investigators shows that the Wolffian canals arise from the pleuro-peritoneal epithelium of the middle plate from which solid cell cords are formed and pass in towards the side of the Wolffian duct. In the higher vertebrates the development of the primitive kidney is to a certain extent abbreviated, in so far as the separate cords of cells which arise at the constricting off of the primitive segments lie very close together and constitute an apparently undifferentiated cell mass out of which the mesonephric tubules subsequently appear to have been differentiated. The source of its material (mesonephros) is either directly or indirectly the epithelium of the body cavity as it has been possible to prove in many cases in Selachians, amphibia and amniota."

(c) *The third view is that the Wolffian tubules are derived independently of previous existing epithelium through differentiation of the Wolffian mesoblastic tissue.* This view was first advanced by Remak (50) and accepted by His (80), Bornhaupt (67), Egli (76), Sernoff (76), Mihalkovics (85) and H. Meyer (90). Balfour (79), Sedgwick (80) and Fürbringer (78) hold this view for that portion of the Wolffian body developing distal from the sixteenth somite. Mihalkovics (85) has made a very thorough and exhaustive study of the development of the Wolffian body in the lizard and chick and finds no evidence whatever to substantiate the views of Braun, Kölliker and Rensen. Mihalkovics has shown that the tubules of chick and lizard which correspond to the seventh to the eleventh somite inclusive arise from the coelom epithelium and that each tubule is connected with the coelom cavity by means of a funnel shaped nephrostome. At the median side of each nephrostome and projecting out from the root of the mesentery is a free glomerulus. It is admitted by all modern investigators that the above constitutes the head-kidney or pronephros which is in no way connected or homologous with the Wolffian body. The error of Balfour, Sedgwick and others arose no doubt from the fact that they regarded the pronephros as the anterior portion of the Wolffian body. I have verified the work of Mihalkovics in the chick and find no nephrostomes or free glomeruli farther distal than the body

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somite. Sedgwick makes the sixteenth somite the point of differentiation. His (80) in description of embryo "a" says that the thickness of the walls of the Wolffian duct at an early period is double the size of that structure later on. This fact would cause him to conclude that the tubules arise from the duct by a fold and a consequent thinning out at this point if the collective evidence of vertebrates did not point to their formation from the Wolffian mesoblast. Nagel (89) in his description of two human embryos rejects the coelom theory in toto and while admitting that his embryos were much too old to give information on this point says if it were not for the opinion of His (see above) he would be inclined to believe that the tubules arise as outgrowths from the Wolffian duct. In order to prove unequivocally that the Wolffian tubules arise from the mesoblastic tissue of the middle plate one must have embryos of such ages which will show the complete cycle of changes from undifferentiated mesoblastic tissue to fully formed Wolffian vesicles. From this point of view I am unfortunate in the selection of my subject material as in my youngest embryos (2.5 mm.) the segmental vesicles are already well formed and differentiated from the surrounding tissue (see w. v. figures 1 and 2). If the vesicles arose from the coelom epithelium one would expect to find some indication of this occurrence at the point to where they were constricted off from the latter, opposite to the vesicles, but by observing the vesicles (w. v.) in figures 1 and 2, it will be seen that no fissures, thinning out of the epithelium, or depression of the latter are to be found.

The anlage of the Wolffian canals develop in a distally extending direction and in the embryo from which figures 1 and 2 are taken the vesicles are well formed at the proximal end. At the distal end of this embryo the cells of the mesoblastic tissue are just arranging themselves around a common center and no lumen is present. Another point which adds considerable confirmatory evidence is the fact that the immature vesicles at the distal end are no nearer the coelom epithelium than the more fully developed vesicles of the proximal end; which should be the case if the vesicles arose from the coelom epithelium. In the embryo (3 mm. long) from which figures 7 and 21 are taken one finds separating the coelom epithelium from the underlying blastem, first a compact layer of connective tissue (c. t.) and second an intercellular space (i. s.) each of which amounts to more than the thickness of a tubule. With the exception of the point at which the tubules adjoin the Wolffian duct the

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coelom epithelium is separated from the underlying structures in this embryo. As previously stated, I admit that my evidence is not complete but all the facts which I found point strongly to the origin of the Wolffian canals from the mesoblastic cells of the middle plate. I hope in the near future to obtain younger embryos which will unequivocally settle this point. While the theories regarding the origin of the anlage of the Wolffian canals are numerous there is a corresponding scarcity of accounts describing the changes by which the primary vesicles are metamorphosed into a fully developed canal, ending distally in a Malpighian body and proximally opening into the Wolffian duct. Sedgwick (80) gives the following account which is decidedly indefinite, "from the inner and dorsal wall of the vesicle a glomerulus is ultimately developed. The whole structure grows enormously and gives rise to the Malpighian body and complicated coils of the later Wolffian tubule. The question as to whether or no there are outgrowths from the Wolffian duct to meet the independently developed Wolffian tubules is not easy to answer. I am not now in a position to give a definite answer and will merely state that there are appearances in my sections which incline me to the opinion that there are outgrowths from the Wolffian duct which in the case of the primary Wolffian tubules are solid but hollow in the case of the secondary and tertiary tubules."

Waldeyer (65) regarded the tubule proper as an outgrowth from the Wolffian ducts while the Malpighian body develops separately in the intermediate cell mass and later joins the tubule. Braun (77) holds in reptiles that there is a short connecting canal given off from the Wolffian duct which joins the segmental vesicle and that by the lengthening out of this canal the tubule proper is developed; while the Malpighian body is formed from the vesicle itself. The most painstaking and the only complete account which I can find is by Mihalkovics (85)and he gives in detail, illustrated by a number of figures, the various changes assumed by the vesicle in its conversion into a Wolffian tubule. He gives an account of this process in both the lizard and the chick and as they agree in all essential points it will serve our purpose to relate briefly a summary of this change occurring in the chick. The Wolffian vesicles are situated at the median side of the Wolffian duct and their contiguous surfaces are in close contact and at the point of union, there is a melting away of the cells and a communication is formed connecting the lumen of both vesicle and duct. At the same time that the above is occurring the round

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form of the vesicle becomes flattened by the sinking in of its dorsal wall and as a result we see in cross section, a half moon shaped body the lateral wall of which is joined to the median side of the Wolffian duct, and its convex wall is ventral and at the median side of the urogenital ridge, close to the coelom epithelium and its median point directed towards the aorta. In the concavity of the half moon is an aggregation of connective tissue which is the anlage of the future glomerulus. The short canal which connects the vesicle with the Wolffian duct is the anlage from which, when fully developed, a tortuous tubule arises; while the Malpighian body alone arises from the half moon shaped Wolffian vesicle.

This account of Mihalkovics for the chick is entirely different from what I have found in Sus. In the pig the Wolffian vesicle assumes an oval form with its long diameter directed dorso-ventralwards, the walls of the Wolffian vesicle and duct being in close contact. Shortly after this the two are connected by a short canal, which is given off from the dorso-median wall of the Wolffian duct and uniting at the dorso-lateral border of the vesicle. In figure 4 the vesicle (w. v.) is seen united to the Wolffian duct (w. d.) by a short curved canal as above described. By comparing figures 1 and 2 with figure 4 it will be seen at this stage that the middle plate has increased considerably in size and now projects into the body cavity and from this period on will be designated as the Wolffian ridge. The vesicle having become oval has receded back from the coelom epithelium (c. e.) and its long diameter is vertical to the body axis. Otherwise the relations of the vesicle to surrounding tissues and organs are not changed from what was described in figures 1 and 2. A lumen in the canal connecting vesicle and Wolffian duct is not present at this early period (4). As to the origin of this canal whether derived from the vesicle or from the Wolffian duct I can not positively state, but it would seem that it is derived from the latter, from the fact that its cells like those of the Wolffian duct have taken the stain with great avidity while the cells lining the vesicle have pale nuclei. By comparing figures 4 and 5 it will be seen that the next stage of development is brought about by the sinking in of the median wall of the vesicle at point 'a' and causes the latter to assume somewhat of an 'S' shape (figure 5) whereby the anlage of the three portions of each tubule and Malpighian body can be differentiated. The proximal portion of the tubule (5) is quite narrow and it now has a distinct lumen and curves dorsally and passes under the ventral border of the cardinal vein (c. v.) and shortly afterwards unites with the

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second portion of the tubule at point 1 (figure 1). The second portion of the tubule extends from 1 to 2 and is spindle shaped(figure 5). This second portion curves ventralwards with a slight lateral deviation and then becomes constricted at point 2, then makes a sharp curve medianwards and passes over into the third portion of the tubule. This third portion extends from point 2 to anlage of the Malpighian body and like the first portion is quite narrow. The third portion is directed medianwards and is parallel with the ventral surface of the Wolffian ridge. The anlage of the Malpighian body is the expanded distal end of the third portion of the tubule (5) and its median surface is in close relation with the aorta (A). In figure 6, a trifle older stage is shown and the several portions of the tubule are more clearly defined than in figure 5. From the preceding account it will be seen that the two distal portions of each tubule and the Malpighian capsule are derived from the Wolffian vesicle. By comparing figures 4, 5 and 6, it will be seen that the lumen of the two distal portions of the tubule and the Malpighian capsule are filled with darkly stained formative cells while in figure 6 no such cells are present in the proximal (first) portion of tubule. This fact is additional evidence that the first portion of the tubule arises as an outgrowth from the Wolffian duct. In figure 8 the first portion of the tubule and the Wolffian duct are also seen to enclose these building cells but I think it purely accidental here and believe they have migrated from the other portions of the tubule, after union with the Wolffian duct; for in figure 3, from a section showing Wolffian vesicles (w.v.) and Wolffian duct, the former are seen to enclose these formative cells while the latter has a clear lumen.

Mihalkovics (85) represents the glomerulus as developing pari passu with the tubule. In Sus this does not seem to be the case. In an embryo of three mm. from sections of which figures 21 and 22 are taken the canals in the proximal three-fourths of the gland have assumed their typical curves, but the expanded distal end of tubule which is the anlage of the Malpighian body (a. m. b.), shows no evidence of invagination. In figure 20, the anlage of Malpighian body shown in figure 22 is seen more highly magnified; it is to be noticed that no evidences of invagination are to be seen. In embryos from 3 to 3.5 mm. the changes relative to the invagination of the Malpighian capsule and the formation of the glomerulus are first to be seen. The origin of the Malpighian tuft of vessels (glomerulus) has, so far as I have been able to ascertain, received very little attention from workers in this field of embryology. The only

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detailed account I have found is by Mihalkovics (85) who accepts the theory advanced by Götte (74) and Fürbringer (78) for amphibia and Braun (77) for reptiles. Mihalkovics found in the chick that the invagination of the Malpighian capsule went on pari passu with the development of the tubule and that first a collection of mesoblastic cells are noticed around the dorsal wall of the capsule and these later are invaginated into the capsule and become the anlage of the glomerulus. At this period no branches are seen approaching the Malpighian body from the aorta. Soon after invagination has occurred, groups of darkly stained cells are to be seen among the connective tissue of the glomerulus anlage. According to Mihalkovics these darker stained cells are first transformed into colorless and then colored blood corpuscles; surrounding connective connective tissue becoming the coiled vessels. Mihalkovics quotes Romiti and Schafer as giving this origin for the blood corpuscles and their enclosing vessel walls, for other organs. I do not doubt the perfect physiological propriety of this view but as a matter of fact it does not occur here. In figure 7, from an embryo 3 mm. long, the changes preparatory to formation of the glomeruli are to be seen. It will be noticed in this figure that the aorta (a) is relatively of large size and that opposite the median point of the Malpighian capsule, there is an evagination of the aorta and at this point a diverticulum is given off from the latter, which passes outwards into the connective tissue of the Wolffian ridge and comes in close relation with the dorsal wall of the Malpighian capsule. The wall of the aorta is continuous with the wall of the diverticulum and the latter is seen to be filled with numerous blood vessels enclosing blood corpuscles. In some cases I find no diverticulum from the aorta, but a number of small blood vessels instead which ramify on the dorsal surface of the capsule; preparatory to invagination of the latter In figure 17, from an embryo of 4 mm. in length the glomerulus is commencing to invaginate while in figure 16 a fully developed Malpighian body, from a 5 mm. embryo, is shown; the glomerulus being entirely invaginated and surrounded by a Malpighian capsule. The cells seen in the glomeruli of figures 16 and 17 are the nuclei of the endothelial cells of the coil vessels, and the wavy outline of the latter is seen in figure 16. In figure 16 in the cells lining the Malpighian capsule the transition from cylindrical to cubical and later to connective tissue is clearly shown. Figure 13 also represents a mature Malpighian body but owing to greater pressure there is less space between glomerulus and capsul thane is seen

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in figure 16. In figures 18 and 19, from an embryo 5 mm. long the Malpighian bodies are fully developed and the large branches given off to the glomeruli from the aorta are seen.

Each fully developed Wolffian canal consists of three typical portions, a dorsal (first), ventral (third) and middle (second) which are connected by two sharp curves. The dorsal portion cylindrical in form affords the connection with the Wolffian duct and then curves dorsalwards along the lateral edge of the Wolffian ridge and then passes medianwards along the ventral edge of the cardinal vein and approaches close to the aorta, on the inner side of the ridge, where it makes a sharp curve and passes over into the spindle shaped middle portion of tubule. The middle portion passes ventralwards and then curves under the first portion and here makes a sharp curve and passes into the anterior portion of the tubule which is directed medianwards and passes close to and almost parallel with the ventral surface of the Wolffian ridge and then expands into the capsule of the Malpighian body, at the median ventral angle of the ridge. The above described relations are readily seen in figures 8 and 12, the first or posterior portion of the tubule extends from the Wolffian duct (w. d.) to point designated (1) where there is a sharp curve. The middle or second spindle shaped portion extends from point (1) to (2) where we find the second sharp curve. The anterior or third portion of tubule extends from point (2) to the Malpighian capsule. In figures 9 and 10 (left section) the proximal two-thirds of first portion of tubule (t. w.) is seen. In figure 12 a complete tubule with its Malpighian body is shown. In figure 8 the tubule is seen arising from the ventral side of the Wolffian duct, an occurrence which I have only found two times in examining several thousand sections of this region. In figure 12 at point (s) (in first portion of the tubule) there is seen a sharp secondary curve. In figure 8 from a somewhat younger embryo this secondary curve is present but less sharply defined. I do not find a description of this secondary curve in the writings of any author who has investigated the Wolffian body. While each Wolffian canal shows three typical positions it is impossible to find any two canals which are identical throughout their entire course. In embryos of 5 mm. from sections of which figures 18 and 19 are taken, it is no longer possible to recognize the entire course of a tubule. As the Wolffian body develops the tubules lengthen out and new curves arise, giving the canals a highly tortuous and convoluted course.

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With the formation of the primary tubules and their glomeruli the growth of the Wolffian body is by no means complete. Two factors contribute to the further growth of this organ; first the lengthening out of the several portions of each tubule, the intensification of the primary curves and by the addition of new ones; second by the formation of secondary, tertiary and quaternary canals. I shall designate as secondary canals all tubules developing subsequent to the primary set. As the origin of the primary mesonephric tubules gave rise to several theories, we have likewise a number of different views regarding the origin of the secondary.

(a) The first view—The secondary tubules and their glomeruli arise either by fission or buds from the primary set. Either of these processes may have as a starting point the wall of the Malpighian capsule or the tubule itself. Braun (77) found in reptiles and Spengel (76) in amphibia that the primary glomeruli are first divided by fissures which continue along the course of the tubule until the Wolffian duct is reached. In Selachians according to the statements of Sedgwick (80) and Balfour (74) the glomeruli is the starting point of proliferation; cell buds grow out from the latter and towards the Wolffian tubules lying in front of them with which their blind ends fuse. After this union has been effected they detach their other end from the parent tissue. Renson (83) held the same view for birds but gives no adequate proof.

In discussing the origin of the secondary canals in the human embryo Nagel (89) says one finds numerous accumulations of epithelial cells in the middle of the sections and which might lead one to think the further growth of the tubules occurs through differentiation of the Wolffian tissues. But the examination of whole series of sections shows most clearly that these epithelial collections stand in direct relation with the previous formed canals and that they represent the solid ends of the same. Nowhere is there to be seen the transition of the cells of the Wolffian tissue to the epithelial cells which would be the case of the latter arose from the former. The solid end pieces of the canals are sharply defined from the surrounding tissues as the canals themselves. From this analysis Nagel concludes that the later development of the Wolffian canals in man occurs through a process of buds or outgrowths of the previously formed canals. Sedgwick (80) in describing this process in the chick does not seem to arrive at a definite conclusion but thinks that the secondary arise from the dorsal walls of the primary set of tubules.

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(b) Second view—The secondary canals arise like the primary from invaginations of the coelom epithelium. Fürbringer (78) is an advocate of this theory and says that the secondary canals arise from the coelom epithelium on the median side of the primary canals and passes into the Wolffian tissue in the form of cell cords which later lose their primary connection.

(c) Third view—The secondary canals and glomeruli arise independently of the primary through a process of differentiation of the Wolffian mesoblast. This view was first advanced by Bornhaupt and later confirmed by Balfour (79) and Mihalkovics (85). My own investigations are in perfect accord with this later view and I will attempt to show that in Sus the secondary canals arise independent of the coelom epithelium and primary tubules, through a differentiation of the mesoblastic cells of the Wolffian ridge. Mihalkovics (85) in reptiles and birds finds no evidence that the secondary canals arise from the primary through fission or buds. According to Sedgwick (80) the secondary canals of the chick arise dorsal from the primary and the tertiary dorsal from the secondary; but Mihalkovics has shown that the secondary canals may arise either ventral, dorsal or intermediate from the primary. Investigations of the origin of the secondary canals in Sus is difficult from the fact that the secondary canals do not appear until the primary are quite fully formed. In figures 8, 21 and 22 the several portions of each tubule are readily seen, no secondary canals have as yet appeared. Like the primary, the secondary canals develop in a proximal-distalward extending direction. In figure 21 from the proximal region of the Wolffian body of an embryo 3 mm. long, I find the first changes which lead up to the formation of the secondary canals; midway between the spindle shaped second portion of the primary canal and the aorta there are to be seen several collections of mesoblastic cells which are closely packed together. These cells take the stain with great intensity and contrast strongly with the surrounding connective tissue. I find no branches from the aorta approaching these groups of cells nor any thickening or invaginations extending in from the overlying coelom epithelium. These cells are at quite a distance from the latter and even though epithelial cords were present it would be difficult to conceive their passage through connective tissue, intercellular spaces and primary tubules and finally reach the designated point in figure 21. In figure 11 from an embryo 4 mm. long we see the next stage in the development of a secondary canal (t. w.); here a base-

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ment membrane is present and the darkly stained mesoblastic cells are assuming a radial arrangement and lumen i just appearing in the vesicle. The shape of this secondary vesicle is oblong, its width being about one-half of its length; while cross sections of primary vesicles are nearly round (figures 1, 2 and 3). The next period of development is also seen in figure 11 where the anlage of a secondary canal is just ventral to the above described vesicle and has assumed somewhat of a ladle shaped form. By comparing the anlage of these two tubules (figure 11) it will be seen that the median portion of the vesicle becomes the anlage of the Malpighian body while the lateral portion becomes the tubule proper. This occurs in much the same way as described in the primary vesicles (figures 4, 5 and 6), although the process of differentiation of the vesicle into a tubule is somewhat abbreviated in the case of the secondary canals. As to the division of a Malpighian body by fissure or buds growing out from it—I have carefully examined the sections of a dozen embryos ranging in size from 3 to 5 mm. and nowhere find evidence of such occurences. In regard to Nagel's view (89) that the secondary canals arise as outgrowths from the primary I can feel sure in saying that it does not occur. One can find numerous sections similar to figure 14 which appear like the outgrowth of a secondary tubule from a primary, but such is not the case for by following this outgrowth in consecutive sections it will be found to continue into a secondary tubule and the apparent blind sack to be caused by a sharp curve which the tubule made before joining the collective portion of the primary canal. According to Mihalkovics (85) secondary canals in the chick are formed either dorsal, ventral or medianwards from the primary. By comparing figures 9, 10 and 21, it will be seen that the first portion of the primary tubule passes very close to the lateral surface of the Wolffian ridge and then curves backward to the cardinal vein and lies directly in front of the ventral surface of the latter. From this it will be seen that there is but little space for secondary canals to develop dorsal from the primary and I have only found one instance of this occurrence which is shown in figure 15. The secondary canals do not arise ventralwards from the primary for a like want of space (figure 9) but are found to develop medianwards from the primary (figure 11). The secondary glomeruli are situated lateral and dorsal from the primary; the latter occupying a position near the inner portion of the gland just dorsal to the germinal epithelium (g. e.). The relations of primary and secondary Malpighian bodies are shown in figure 10.

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In the chick of five or six days Mihalkovics finds from 12 to 18 Wolffian tubules opening into the Wolffian duct in each body somite and that it is no uncommon occurrence to find three tubules emptying into the duct in the same section and besides the tubules which open direct into the Wolffian duct he finds from 20 to 40 indirect tubules in each somite. These indirect tubules empty into the collective (first) portion of a direct canal. This would make a total of from thirty to sixty direct and indirect tubules for each body somite. I find in Sus from 2 to 3 tubules emptying into the Wolffian duct in each body somite. In embryos of four, five, eight and fifteen mm. respectively the number of direct canals remains practically the same, that is two to three to each somite. In embryos of four to five mm. length (figures 10, 11–18, 19) from two to three Malpighian bodies are to be seen in each section in the middle two-thirds of the Wolffian body. In embryos ranging in size from 8 mm. to 1-5 10 cm. one frequently finds from six to eight Malpighian bodies in a single section. From this one naturally comes to the conclusion that all or nearly all of the secondary canals in Sus are indirect; emptying into the collective portion of a primary canal. The examination of a number of sections demonstrates the correctness of this as can be seen in figures 14 and 19. In figure 11 the proximal end of the anlage of a secondary canal is in contact with the median wall of a primary tubule and later will open into it. I have only found one instance in which two tubules open into the Wolffian duct in the same section. This is shown in figure 15, the outer of the two tubules being a secondary while the inner is a primary one. Thus it appears that an occasional secondary tubule opens directly into the Wolffian duct, but is quite a rare occurrence.

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LIST OF REFERENCE LETTERS

a.....aorta
amb.....anlage Malpighian body
c.....coelom or body cavity
ca.....capsule
ce.....coelom epithelium
ch.....notochord
ct.....connective tissue
e.....epiblast
g.....glomerulus of the Malpighian body
ge.....genital epithelium
gr.....genital ridge
is.....intercellular space
h.....hypoblast
mb.....Malpighian body
mc.....medullary canal
mes.....mesentery
mp.....middle plate
ms.....mesoblastic somite
m.....mesoblast
ns.....spinal chord
so m.....somatopleuric layer of mesoblast
sp m.....splanchnopleuric layer of mesoblast
ti.....intestine
tw.....Wolffian tubule
tw^1.....primary Wolffian tubule
tw^2.....secondary Wolffian tubule
vc.....Cardinal vein
vs.....spermatic vein
wd.....Wolffian duct
wv.....Wolffian vesicle
wr.....Wolffian ridge

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EXPLANATION OF PLATE XVII

Fig. 1. Cross section form the proximal end of the Wolffian body of an embryo 2.5 mm. long. III—4 × 100.

Fig. 2. Left side of figure 1 more highly magnified I—5 × 190.

Fig. 3. An oblique section passing through the distal end of a 3 mm. embryo. The Wolffian duct and three Wolffian vesicles are shown. III—5 × 280.

Figs. 4 and 5. Cross sections from the distal end of a 4 mm. embryo. I—5 × 190.

EXPLANATION OF PLATE XVIII

Fig. 6. Cross section from the distal end of a 4 mm. embryo. I—5 × 190.

Fig. 7. Cross section from the middle third of the Wolffian body of a 3 mm. embryo. III—4 × 100.

Fig. 8. Cross section through the middle third of the Wolffian body of a 3 and 5-10 mm. embryo. III—3 × 140.

EXPLANATION OF PLATE XIX

Fig. 9. Cross section through the proximal end of Wolffian body of a 4 mm. embryo. I—5 × 190.

Fig. 10. Cross section through Wolffian bodies of middle third of a 4 mm. embryo 1—3 × 66.

EXPLANATION OF PLATE XX

Fig. 11. Cross section through the middle third of the Wolffian body of a 4 mm. embryo. IV—3 × 125.

Fig. 12. Cross section through the distal end of a 5 mm. embryo. IV—3 × 125.

Fig. 13. Shows the Malpighian body seen in fig. 12 more highly magnified. III—5 × 280.

Fig. 14. Cross section through the middle third of Wolffian body of a 4 mm. embryo, showing Wolffian duct and proximal portions of Wolffian tubules. I—5 × 190.

EXPLANATION OF PLATE XXI

Fig. 15. Cross section through the middle third of Wolffian body of a 4 mm. embryo, showing Wolffian duct and proximal portions of Wolffian tubules. I—5 × 190.

Fig. 16. Cross section through a fully developed Malpighian body of a 5 mm. embryo. I—5 × 190.

Fig. 17. Cross section through a Malpighian body in which the glomerulus is undergoing invagination from a 4 mm. embryo. I—7 × 300.

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EXPLANATION OF PLATE XXII

Fig. 18. Cross section through middle third of the Wolffian body of a 5 mm. embryo. × 160.

Fig. 19. Cross section through the distal end of a Wolffian body of a 5 mm. embryo. I—4 × 39.

EXPLANATION OF PLATE XXIII

Fig. 20. Anlage of Malpighian body before invagination of capsule has occurred. From an embryo of 3 mm. III—5 × 280.

Fig. 21. Cross section through the proximal end of Wolffian body of a 3 mm. embryo. IV—3 × 125.

Fig. 22. Cross section through the middle third of the Wolffian body. From the ame embryo as Fig. 21. III—4 × 125.

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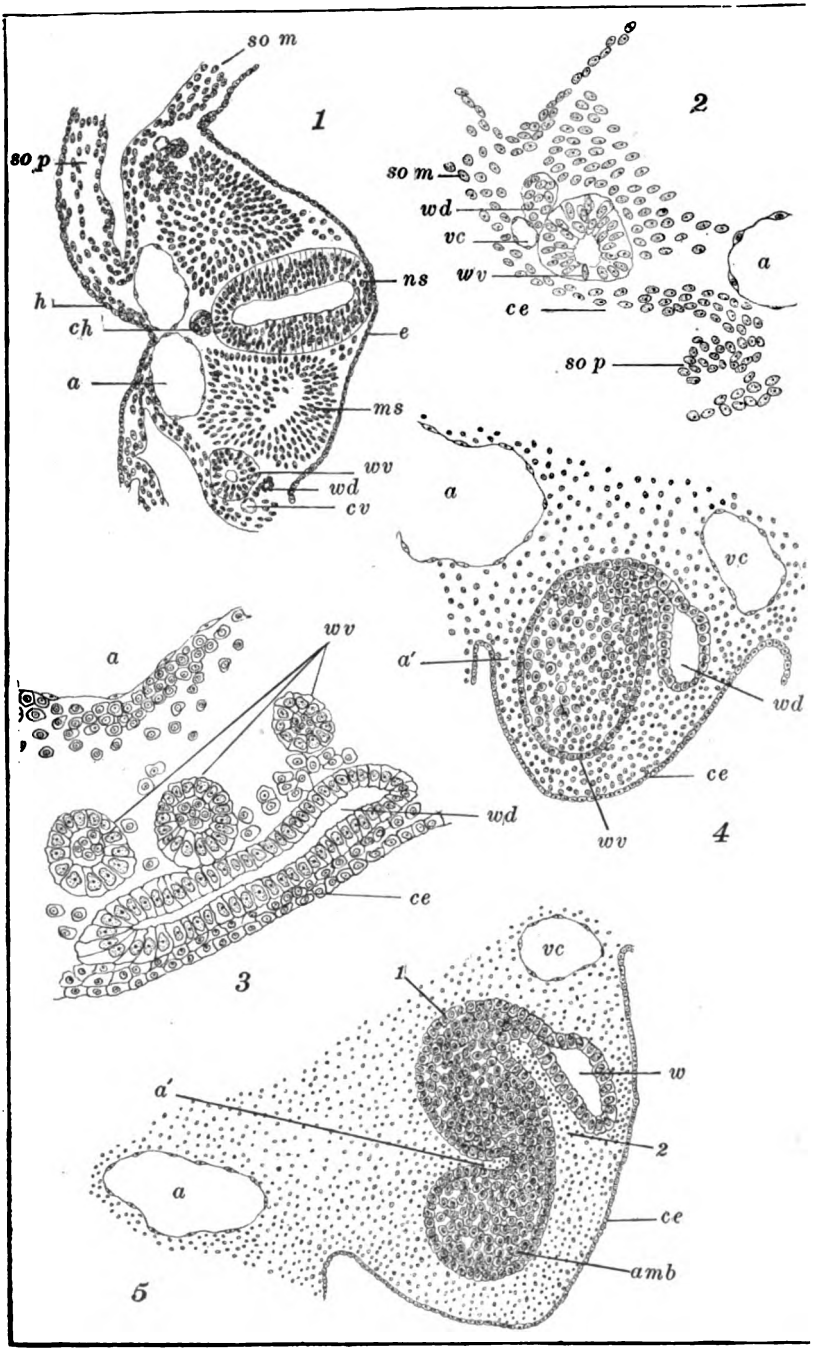

PLATE XVII

ANGLE

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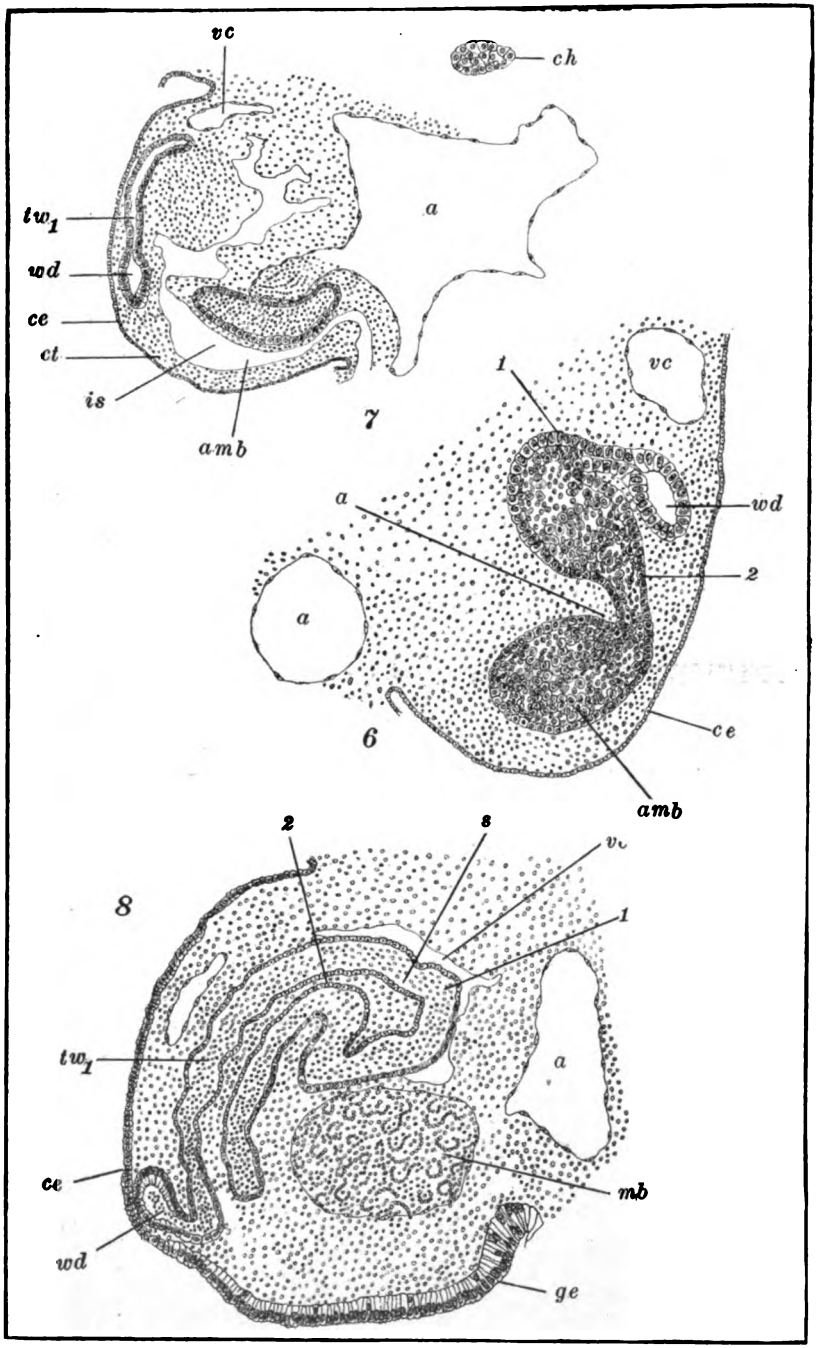

PLATE XVIII

ANGLE

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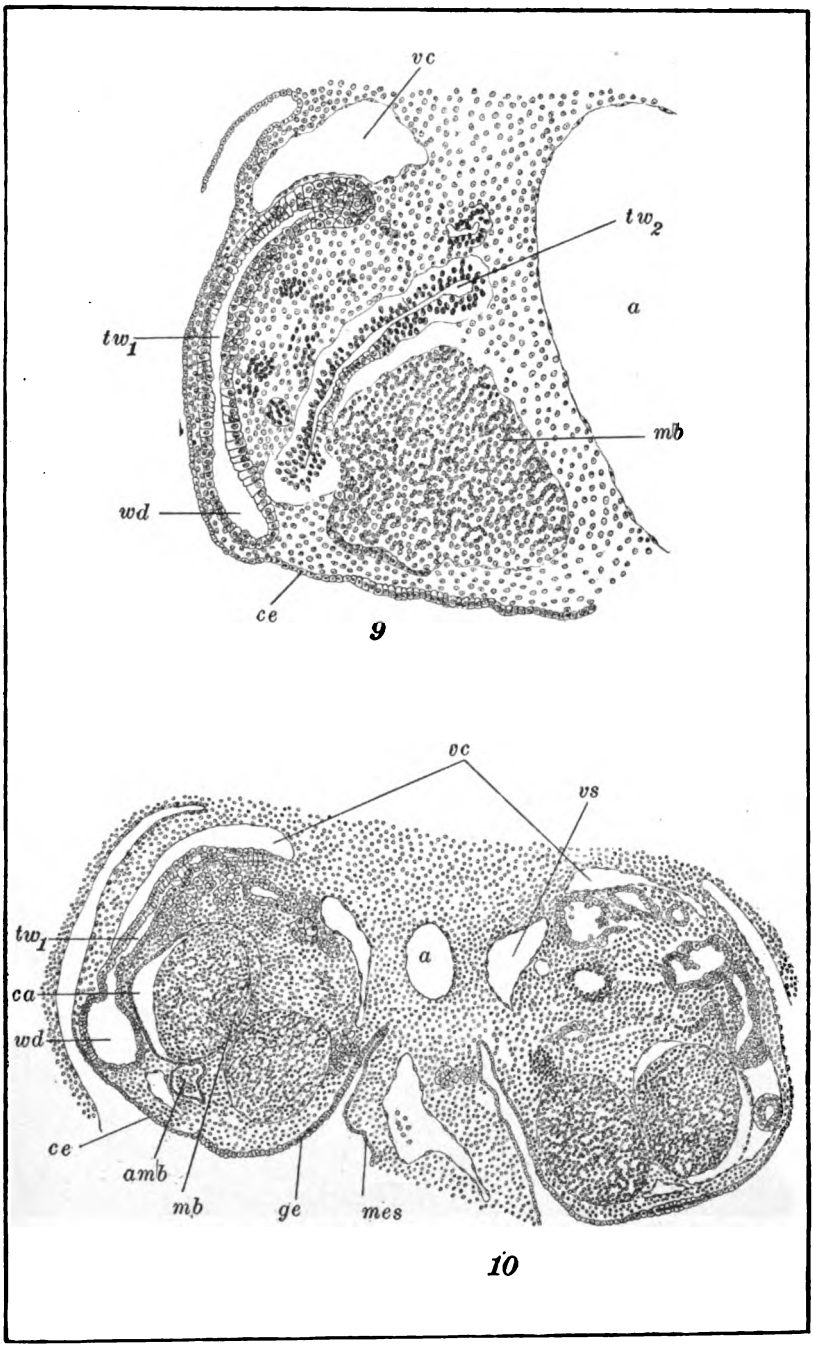

PLATE XIX

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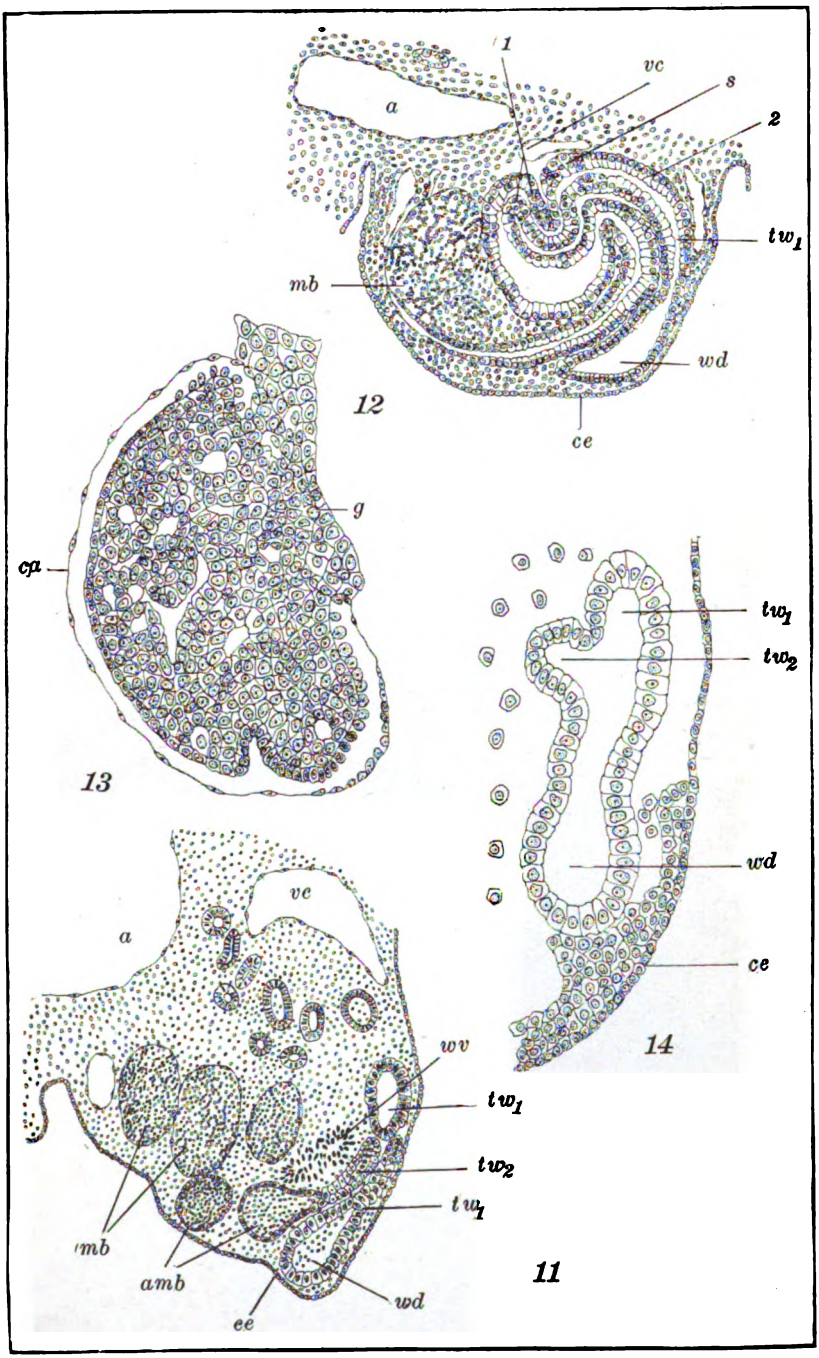

PLATE XX

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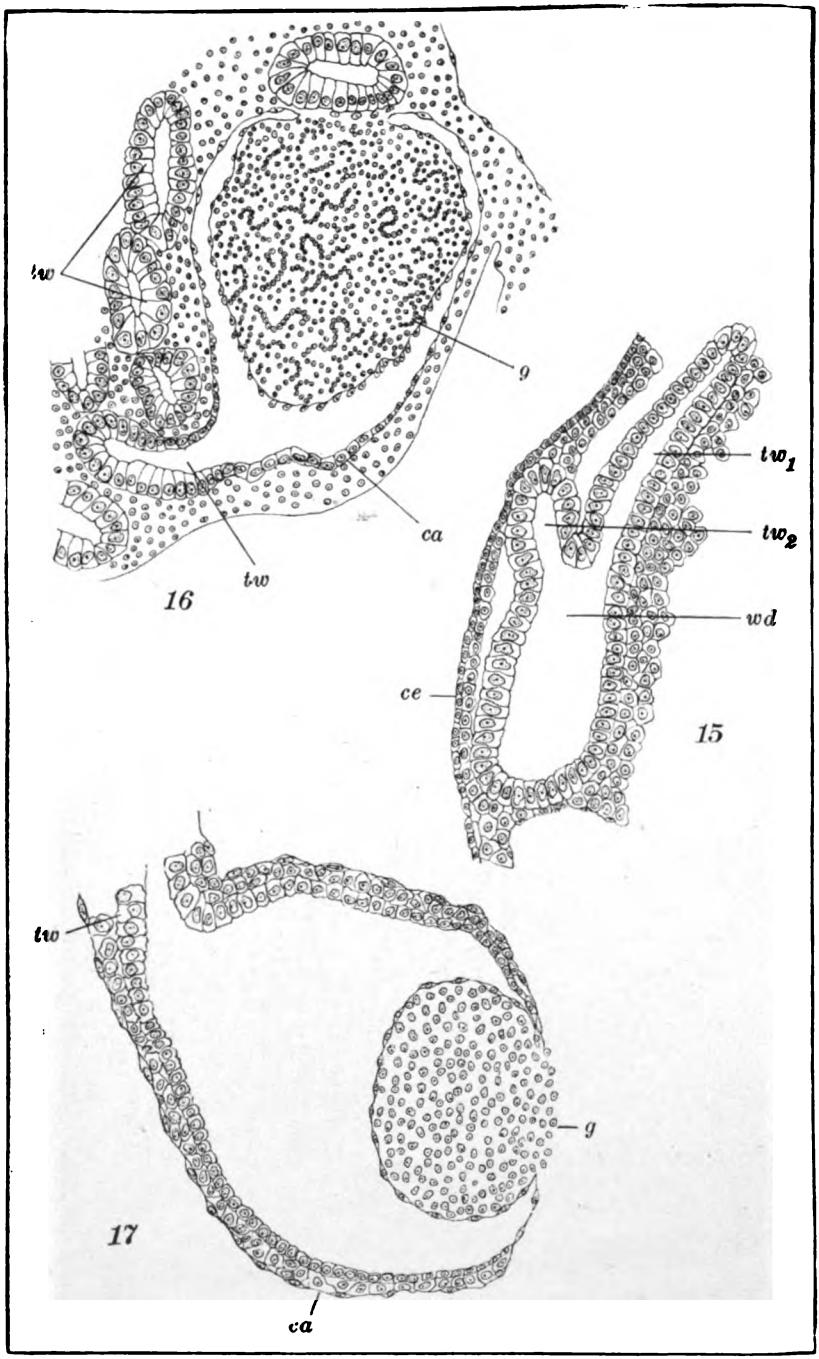

PLATE XXI

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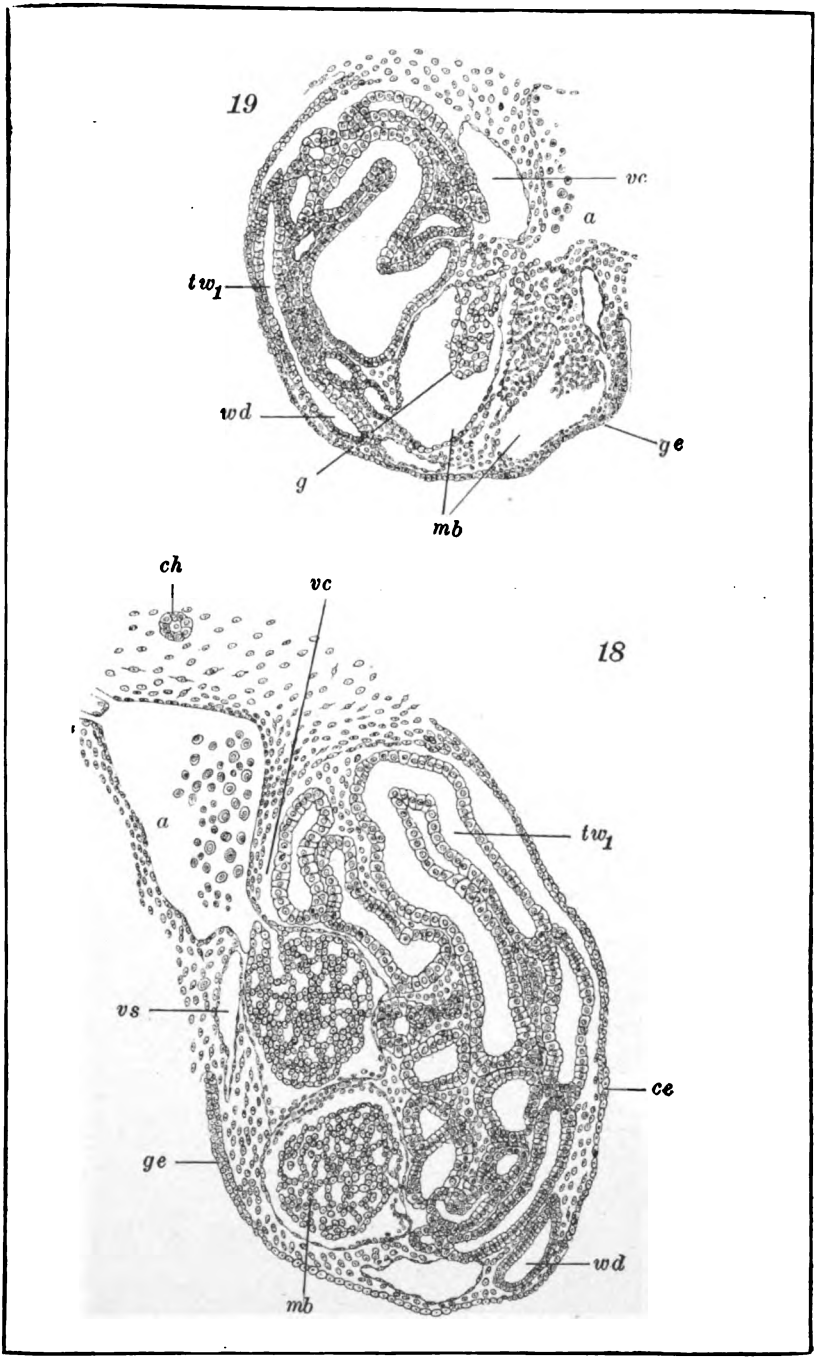

PLATE XXII

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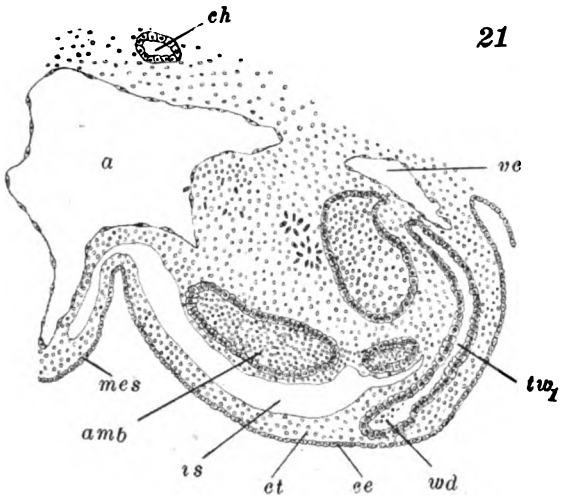

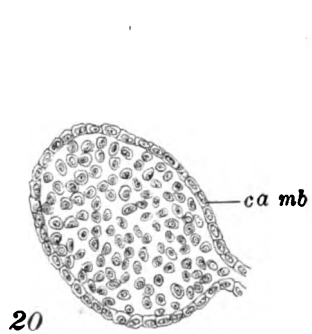

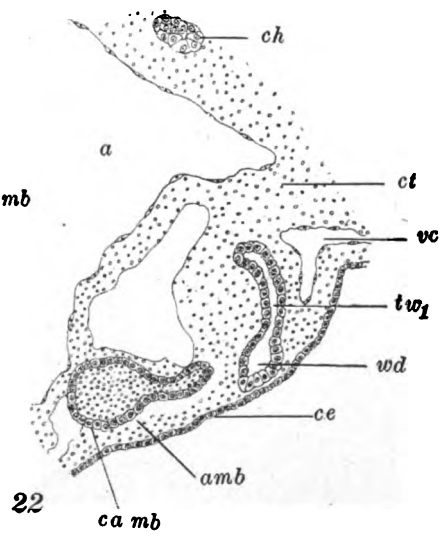

PLATE XXIII

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VARIATION IN THE HORIZONTAL DISTRIBUTION OF PLANKTON IN DEVILS LAKE, NORTH DAKOTA

ERIK G. MOBERG[1]

The horizontal distribution of plankton has been studied by several investigators and varied results have been obtained. It is usually held, however, that under uniform physical conditions the distribution of the plankton is uniform. The question is of great importance since in quantitative plankton investigations the amount of plankton found at a certain station is usually taken as representative of a large area.

For several years plankton studies have been carried on at the Biological Station at Devils Lake, North Dakota, under the direction of Dr. R. T. Young, and in connection with these studies collections were made during the summer of 1914, to determine whether the organisms in Devils Lake showed any diurnal movements. For this purpose collections were made shortly after noon, just after sunset, and shortly before sunrise from the surface, the 0.6 m., the 2.1 m., and the 3.6 m. levels, the depth of the lake at that place being about 4 m. All the samples were taken in identically the same place and on all occasions the velocity of the wind and the condition of the sky were similar. The samples, each of 500 cc., were concentrated to 10 cc., and counted according to the Sedgwick-Rafter[2] method. In each case the total number of individuals in two or three cells, and therefore in 100 cc. or 150 cc. of the original sample, were counted. The results obtained are shown in Table I.

TABLE I

SHOWING THE NUMBER OF INDIVIDUALS PER LITER OF WATER

	1:00–2:30 P.M.		8:30–9:30 P.M.		3:00–4:00 A.M.	
Depth	Crustacea	Rotifera	Crustacea	Rotifera	Crustacea	Rotifera
Surface	40	310	110	770	160	480
0.6 m.	20	440	90	920	240	560
2.1 m.	25	380	95	680	280	360
3.6 m.	125	330	270	940	260	580
Total all levels.	210	1460	565	3310	940	1980

[1] Numerals refer to notes beginning on p. 265.

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Even if there had been a vertical movement of the organisms the total number of individuals of one series should be approximately equal to that of another series. Instead we find that the evening series contains more than twice as many crustaceans as the noon series, and that collected in the morning more than four times as many. In the case of the rotifers the variations are not quite as large. These results seem to show that there had been horizontal movements of the plankton animals during the intervals between the periods of collecting.

To test the horizontal distribution further and more directly several series of collections were made during the summers of 1914, 1915 and 1916. In some cases the samples were taken from a number of nearby points in a part of the lake where the physical conditions do not vary appreciably, while in others the entire series was collected from a fixed point at short intervals of time. The accompanying maps show the main part of the lake and the approximate locations of the stations at which the samples were obtained.

In connection with some of the 1915 series the amount of phytoplankton and of dissolved chemicals (oxygen, free and albuminoid ammonias, and CO^3 and HCO^3 ions) were determined[3] in order to show the relation between the zooplankton and the food and chemical constituents in the water.

During 1914 and 1915 the Sedgwick–Rafter method of concentrating and counting was used in the plankton work, ten squares being counted in the case of the plants, but in the case of the animals the entire number of individuals in five cells (one-half of the collection) was counted. Except where specified the volume of each sample was 500 cc. and in all cases it was concentrated to 10 cc. During 1916 the collections were made by means of a pump and a plankton-net. The water was measured by means of a water meter and pumped thru the net where the organisms were retained. Five gallons (18927 cc.) were collected and concentrated to 18.9 cc., making a ratio of 1000 to 1. In five cells of each sample the entire number of zooplanktonts was counted.

The results of the measurements and of the analyses are expressed as follows: depths in meters; temperatures in degrees Centigrade; oxygen in cubic centimeters per liter; ammonias as parts per million of nitrogen; carbonates as parts per million of CO^3 or HCO^3 ions; Nodularia (the only filamentous alga) in number of millimeters per liter; other algae (including Coelospherium, Gomphospheria, Dictyospherium, Chroococcus,

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Merismopedia, and a number of others, less common), in standard units[4] per liter; diatoms, rotifers, and crustaceans in number of individuals per liter.

The variation in the horizontal distribution of the plankton may be studied from three different points of view; namely: (1) the variation of the total amount of plankton, (2) the variation of each species, and (3) the correlation between the zooplanktonts, the phytoplanktonts, and the physical and chemical conditions of the environment.

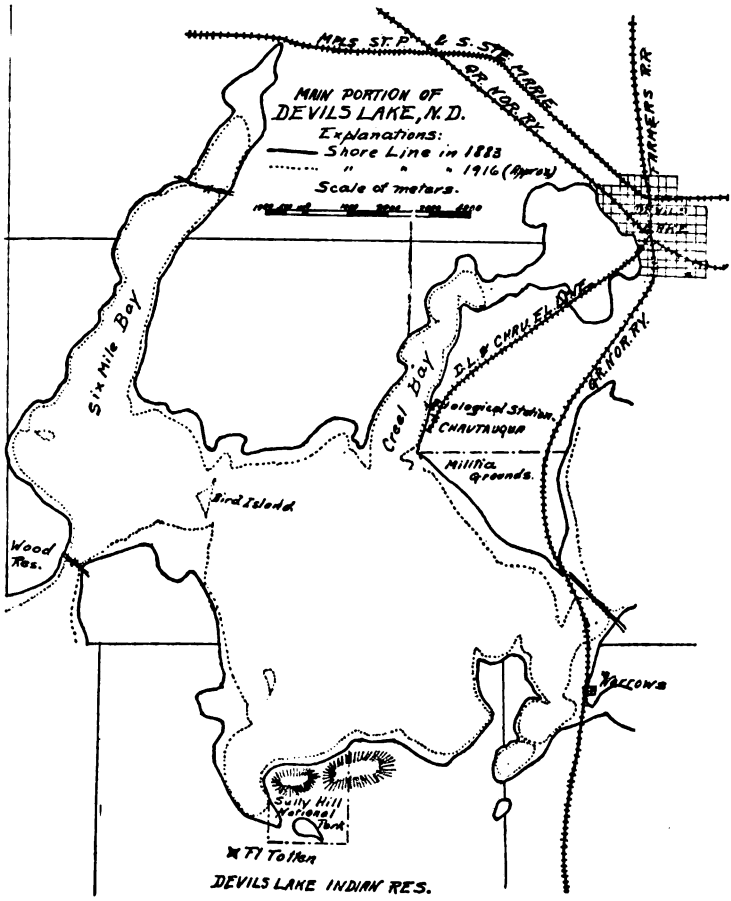

FIGURE 1

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The Sedgwick–Rafter method hardly lends itself to the study of the total amount of plankton but it may be roughly estimated by considering the occurrence of the more important species. The number of individuals of the different species, however, may be determined quite closely.

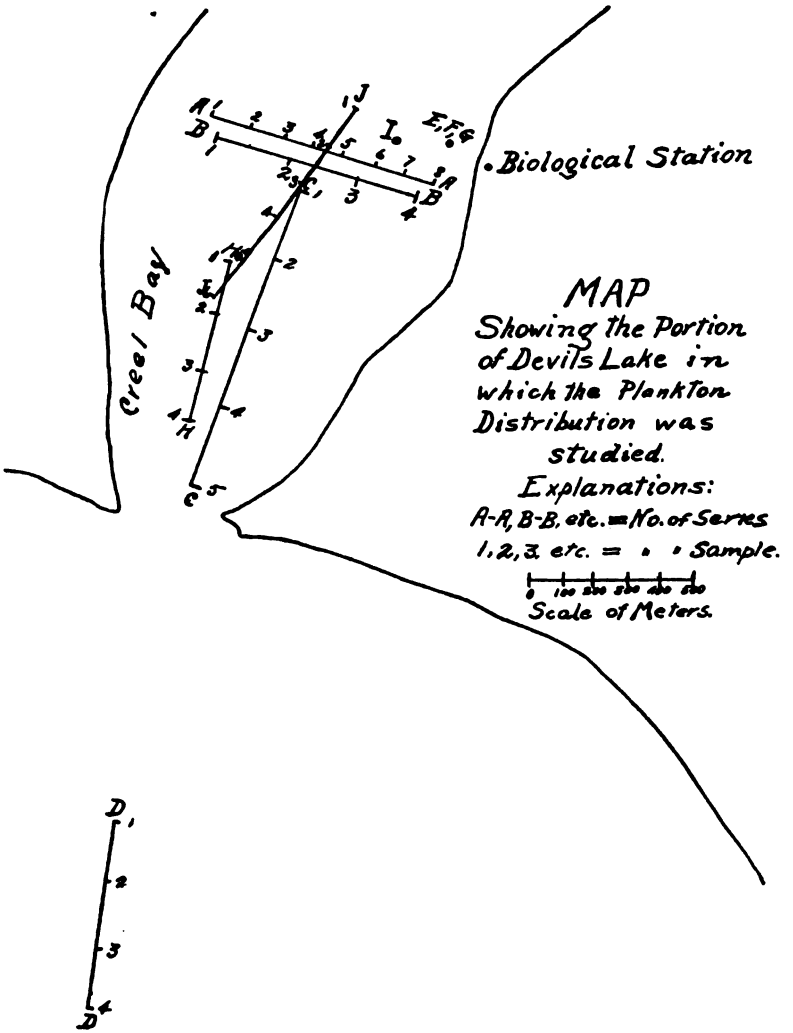

FIGURE 2

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Whipple[5] states that the experimental error is not more than about ten per cent. In the case of some of the algae, the error is probably larger, especially since only ten squares were counted, and in considering the results this should be remembered; but for the animals it is probably less since a large portion of the sample was examined. Since in most cases the depth and the temperature were measured and the amount of chemicals determined, the results give some conception of the relation between the plankton and the environment. It must be remembered, however, that some of the variation in the chemicals is due to experimental error.

Notes on the Different Collections and Tabulation of Results

Series A. A set of eight samples was collected on August 19, 1914 from points lying in a straight line between the two shores of Creel Bay The distance between each two points was about 100 meters and the time required for the whole series was about a half hour. The greatest depth between the two shores was 4.5 meters at points 5 and 6. At no point does the depth vary more than one meter and the character of the bottom is uniform, the points 1 and 8 being outside the littoral zone. At the time of collecting the sky was clear and there was almost no wind. Only the animals were counted and the results are shown in Table II.

TABLE II

Showing Data for Series A

(Amt. = Amount. % var. = variation from the mean in per cent)

	Sample 1		Sample 2		Sample 3		Sample 4	
	Amt.	% var.	Amt.	% var.	Amt.	% var.	Amt.	% var.
Temperature	21.8	+3.3	21.5	+1.9	21.0	−0.4	21.0	−0.4
Brachionus satanicus	108	−39.7	48	−73.2	128	−28.4	140	−21.8
Brachionus mülleri	44	+62.9	40	+48.1	36	+33.3	24	−11.1
Pedalion	248	−32.6	540	+46.7	300	−18.5	324	−11.9
Moina	104	−13.3	40	−66.6	16	−86.6	92	−23.3
Cyclops	16	+10.3	4	−69.2	4	−69.2	24	+84.6
Diaptomus	0	−100.0	4	+100.0	8	+300.0	0	−100.0
Nauplii	80	+66.6	36	−25.0	28	−41.7	48	0.0

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TABLE II (*Continued*)

	Sample 5		Sample 6		Sample 7		Sample 8		
	Amt.	% var.	Amt.	% var.	Amt.	% var.	Amt.	% var.	Mean
Temperature	20.8	−1.4	20.8	−1.4	21.0	−0.4	21.8	+3.3	21.1
Brachionus satanicus	356	+98.9	292	+63.1	200	+11.7	160	−10.5	179.0
Brachionus mülleri	16	−40.7	36	+33.3	4	−85.1	16	−40.7	27.0
Pedalion	564	+53.2	372	+1.1	372	+1.1	224	−39.1	368.0
Moina	44	−63.3	148	+23.3	144	+20.0	372	+210.0	120.0
Cyclops	24	+84.6	28	+93.1	4	−69.2	12	−7.6	14.5
Diaptomus	4	+100.0	0	−100.0	0	−100.0	0	−100.0	2.0
Nauplii	84	+75.0	48	0.0	16	−66.6	44	−8.3	48.0

These analyses show a large variation of all the species and especially of the Crustacea. The total number of animals is almost constant, however, since one form is numerous where another is scarce. The temperature varies one degree but does not seem to have any effect on the number of animals. The variations shown by the different species are summarized in Table III.

TABLE III

PERCENT OF VARIATION FROM MEAN OF SERIES A

	Mean	Maximum	Minimum	Range
Brachionus satanicus	±43.4	+98.9	−73.2	172.1
Brachionus mülleri	±44.4	+62.9	−85.1	148.0
Pedalion	±25.5	+53.2	−39.1	92.3
Moina	±63.3	+210.0	−86.6	296.6
Cyclops	±61.0	+93.1	−69.2	162.3
Nauplii	±35.4	+75.0	−66.6	141.6

Series B. These samples were collected on August 25, 1914, in the same locality and under the same weather conditions as those of series A, but the distances between the different points of series B were about twice as large, and 1000 cc., instead of 500 cc., were concentrated. No separate counts were made of the different species but the animals are grouped under Crustacea and Rotifera. The Crustacea include: Moina,

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Diaptomus, Cyclops, and Copepod Nauplii. The Rotifera include: *Brachionus satanicus, B. milleri*,[6] Pedalion, and a few Asplanchna. The results are shown in Table IV.

TABLE IV

SHOWING DATA FOR SERIES B

(Amt. = amount. % var. = variation from mean in per cent)

	Sample 1		Sample 2		Sample 3		Sample 4		
	Amt.	% var	Amt.	% var.	Amt.	% var.	Amt.	% var.	Mean
Temperature	15.8		15.8		15.8		15.2		
Crustacea	198	−44.7	355	−0.8	552	+54.2	326	−8.9	358
Rotifera	162	−46.2	427	+41.8	316	+4.9	298	−1.0	301

For the Rotifera the mean variation from the average is ±23.4% the maximum variation +42%, and the minimum variation −45.7%, making a range of 87.7%. For the Crustacea the figures are: mean ±27.1%, maximum +54.3%, minimum −44.6%, range 98.9%. Here again the crustaceans show a larger variation than the rotifers, altho the former are more numerous.

Series C. This series was collected on June 6, 1915, from points lying in a straight line parallel to the shores of Creel Bay, point 1 lying just south of where the collections of series A and B were made, and point 5 a short distance inside the mouth of the bay. The sky was clear, the time required about one hour, and the distance between each two points about 250 meters. The results are shown in Table V.

The very slight variation in depth shows no effect upon the organisms. The chemicals, excepting the ammonias, show a uniform distribution, the variations not being greater than the errors of sampling and analysing. None of the plankton forms, nor the plankton as a whole, show any relation to the amount of ammonias. It may be noted that most of the forms were scarce at point 1, and abundant at point 5, but at the intervening points the total amount of plankton appears quite constant. Table VI summarizes the variation of the different plankton forms.

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TABLE V

DATA FOR SERIES C

(Abbreviations as in previous tables)

	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		
	Amt.	% var.	Amt.	% var.	Amt.	% var.	Amt.	% var.	Amt.	% var.	Mean
Depth	4.6	−9.8	4.6	−9.8	5.2	+1.9	5.5	+7.8	5.5	+7.8	5.1
Free Ammonia	0.135	+3.0	0.126	−3.8	0.076	−42.0	0.190	+45.0	0.130	−0.8	0.131
Albuminoid Ammonia	0.96	−15.0	1.76	+55.8	0.81	−28.3	1.13	0.0	1.00	−11.5	1.13
Oxygen	6.2	.....	6.2	.....	6.3	.....	.....	.....	6.2	.....	.....
CO_3 ion	266	+9.7	272	+12.2	213	−12.2	213	−12.2	248	+2.3	242.4
HCO_3 ion	601	−2.4	590	−4.2	642	+4.2	642	+4.2	604	−1.9	616
Nodularia spumigena	86,000	+1.4	164,000	+93.4	46,000	−45.7	70,000	−17.5	58,000	−31.6	84,800
Other Algae	182,000	−49.2	456,000	+27.3	218,000	−39.1	374,000	+4.5	558,000	+55.9	358,000
Brachionus satanicus	12	−53.1	20	−21.9	24	−6.3	32	+25.0	40	+56.2	25.9
Brachionus mülleri	20	+66.6	8	−33.3	12	0.0	16	+33.3	4	−66.6	12
Pedalion fennicum	128	−41.3	168	−22.9	328	+50.5	196	−10.1	272	+24.8	218
Asplanchna sylvestri	0	−100.0	12	−6.2	16	+25.0	8	−37.5	28	+118.7	12.8
Cyclops viridis	12	−40.0	16	−20.0	28	+40.0	8	−60.0	36	+80.0	20
Diaptomus sicilis	96	−11.1	180	+66.6	88	−18.5	72	−33.3	104	−3.7	108
Nauplii	60	−45.6	160	+44.9	152	+37.7	108	−2.1	72	−34.7	110.4

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TABLE VI

PERCENT OF VARIATION FROM MEAN OF SERIES C

	Mean	Maximum	Minimum	Range
Nodularia	±37.9	+93.4	−45.7	139.1
Other Algae	±35.2	+55.9	−49.2	105.1
Brachionus satanicus	±32.5	+56.2	−53.1	109.3
Brachionus mülleri	±40.0	+66.6	−66.6	133.2
Pedalion	±29.9	+50.5	−41.1	91.8
Asplanchna	±57.5	+118.7	−100.0	218.7
Cyclops	±48.0	+80.0	−60.0	140.0
Diaptomus	±26.6	+66.6	−33.3	99.9
Nauplii	±33.0	+44.9	−45.6	90.5

Series D. This series was collected on June 21, 1915, well out in the main part of the lake as shown on the map. A very slight south-west wind was blowing and the sky was clear. The time required was about one hour and the distance between two points about 200 meters. Table VII shows the results.

These analyses show a uniformity of physical and chemical conditions, except in the case of the free ammonia which varies to an unusual extent. Since it is present in small amounts it is probable that the greater part of its variation is due to experimental error. No relation is shown between the amount of ammonia and the amount of plankton. All the animals, and especially the adult crustaceans, occur in small numbers, so that some of them will be excluded in tabulating the variation percentages. It is important to note that at point 3 all animals, except Cyclops and the nauplii, are absent, while at point 4 most of them are quite numerous. The summary of the variations is shown in Table VIII.

TABLE VIII

PERCENT OF VARIATION FROM THE MEAN OF SERIES D

	Mean	Maximum	Minimum	Range
Nodularia	±51.9	+61.2	−72.1	133.3
Other Algae	±11.1	+22.3	−14.6	36.9
Brachionus satanicus	±138.1	+276.2	−100.0	376.2
Brachionus mülleri	±81.8	+118.2	−100.0	218.2
Pedalion	±57.1	+77.1	−100.0	177.1
Nauplii	±50.0	+100.0	−80.0	180.0

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TABLE VII

DATA FOR SERIES D

(Abbreviations as in previous tables)

	Sample 1		Sample 2		Sample 3		Sample 4		
	Amt.	% var.	Amt.	% var.	Amt.	% var.	Amt.	% var.	Mean
Depth	5.8	+1.8	5.8	+1.8	5.6	−1.8	5.5	−3.5	5.7
Temperature	20.0	.....	20.0	.....	20.3	.....	20.3	.....	.....
Oxygen	6.46	−0.9	6.55	+0.5	6.55	+0.5	.....	.....	6.52
Free Ammonia	0.20	+100.0	0.10	0.0	0.06	−40.0	0.06	−40.0	0.10
Albuminoid Ammonia	0.94	+8.0	0.91	+4.6	0.80	−8.0	0.84	−3.4	0.87
CO_3 ion	226	−4.2	236	0.0	243	+3.0	239	+1.3	236
HCO_3 ion	608	−0.5	585	−4.3	581	−4.9	670	+9.6	611
Nodularia spumigena	44,000	−31.8	104,000	+61.2	92,000	+42.6	18,000	−72.1	64,500
Other Algae	152,000	−3.2	192,000	+22.3	150,000	−4.5	134,000	−14.6	157,000
Brachionus satanicus	16	−84.2	8	−92.1	0	−100.0	380	+276.2	101
Brachionus mülleri	24	+118.2	4	−63.6	0	−100.0	16	+45.4	11
Pedalion fennicum	60	−14.3	96	+37.1	0	−100.0	124	+77.1	70
Asplanchna sylvestri	0	+100.0	0	−100.0	0	−100.0	4	+300.0	1
Cyclops viridis	0	−100.0	0	−100.0	12	+200.0	4	0.0	4
Diaptomus sicilis	16	+300.0	0	−100.0	0	−100.0	0	−100.0	4
Nauplii	20	0.0	40	+100.0	4	−80.0	16	−20.0	20

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Series E. This series was collected on July 27, 1915, from an anchored raft some distance from the shore, where the depth was about four meters. The four samples were taken at fifteen minute intervals beginning at 11:30 A.M. and continuing until 12:15 P.M. The wind was blowing from the south causing small waves, which increased in size toward the end of the series. Cloudiness and sunshine alternated at short intervals thruout the period. The results of the analyses are shown in Table IX.

The temperature and the chemicals are almost constant. The first sample shows a small amount of both plants and animals while in sample 4 the zooplanktonts are abundant. Table X summarizes the percentages.

TABLE X

PERCENT OF VARIATION FROM MEAN OF SERIES E

	Mean	Maximum	Minimum	Range
Nodularia	±10.8	+10.5	−21.5	32.0
Other Algae	±7.0	+14.0	−9.0	23.0
Chaetoceros	±34.7	+36.1	−42.9	79.0
Other diatoms	±69.5	+139.0	−65.9	204.9
Brachionus satanicus	±39.3	+78.6	−41.2	119.8
Brachionus mülleri	±28.2	+54.3	−54.3	108.6
Pedalion	±17.8	+35.6	−16.3	51.9
Cyclops	±25.0	+50.0	−50.0	100.0
Diaptomus	±50.0	+75.0	−100.0	175.0
Nauplii	±55.2	+73.1	−70.1	143.2

Series F and G. These two series were collected on August 3, 1915, at the same point as was series E. Series F represents samples taken from the surface, while the samples of series G were taken from a depth of three meters. The samples of the two series were taken alternately at fifteen minute intervals, the period between the collecting of two samples of the same series therefore being a half hour. The first collection was made at 2:00 P.M. The sky was clear and there was almost no wind. The results of the analyses of series F are shown in Table XI.

These analyses show the physical and chemical factors to be quite constant, and the total amount of plankton seems fairly evenly distributed, except in 1 where all the animals and most of the plants are absent or few in number. The results are summarized in Table XII.

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TABLE IX

DATA FOR SERIES E

(Abbreviations as in previous tables)

	Sample 1		Sample 2		Sample 3		Sample 4		
	Amt.	% var.	Amt.	% var.	Amt.	% var.	Amt.	% var.	Mean
Oxygen	.....	.....	.....6.36	+1.1	6.16	−2.1	6.37	+1.1	6.29
Free Ammonia	0.04	0.0	0.04	0.0	0.04	0.0	0.04	0.0	0.04
Albuminoid Ammonia	1.00	+5.2	0.90	−5.2	0.90	−5.2	1.00	+5.2	0.95
CO_3 ion	258	+4.9	233	−5.3	251	−2.0	243	−1.2	246
HCO_3 ion	590	+0.9	598	+2.2	576	−1.5	574	−1.9	585
Nodularia spumigena	112,000	−21.5	152,000	+6.2	150,000	+4.9	158,000	+10.5	143,000
Other Algae	174,000	−4.7	182,000	−0.3	208,000	+14.0	166,000	−9.0	182,500
Chaetoceros elmorei	100,000	+36.1	54,000	−26.5	98,000	+33.3	42,000	−42.9	73,500
Other Diatoms	98,000	+139.0	20,000	−51.2	32,000	−21.9	14,000	−65.9	41,000
Brachionus satanicus	332	−11.2	276	−26.2	668	+78.6	220	−41.2	374
Brachionus mülleri	84	−54.3	180	−2.2	188	+2.2	284	+54.3	184
Pedalion fennicum	200	−16.3	224	−6.3	208	−13.0	324	+35.6	239
Cyclops viridis	12	−50.0	36	+50.0	24	0.0	24	0.0	24
Diaptomus sicilis	0	−100.0	16	0.0	28	.+75.0	20	+25.0	16
Nauplii	92	+37.3	40	−40.3	20	−70.1	116	+73.1	67

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TABLE XI

DATA FOR SERIES F

(Abbreviations as in previous tables)

	Sample 1		Sample 3		Sample 5		Sample 7		
	Amt.	% var.	Amt.	% var.	Amt.	% var.	Amt.	% var.	Mean
Temperature	20.5	−1.9	20.5	−1.9	21.3	+1.9	21.3	+1.9	20.9
Oxygen	6.3	0.0	6.2	−1.6	6.3	0.0	6.4	+1.6	6.3
Free Ammonia	0.02	0.0	0.02	0.0	0.02	0.0	0.02	0.0	0.02
Albuminoid ammonia	0.40	−20.0	0.40	−20.0	0.60	+20.0	0.60	+20.0	0.50
CO_3 ion	179	−12.3	195	−4.4	214	+4.9	228	+11.8	204
HCO_3 ion	674	+4.7	665	+3.3	631	−2.0	608	−5.6	644
Nodularia spumigena	296,000	+44.4	190,000	−7.3	160,000	−21.9	174,000	−15.1	205,000
Other Algae	90,000	−37.1	182,000	+27.2	162,000	+13.3	138,000	−3.5	143,000
Chaetoceros elmorei	30,000	−78.3	130,000	−5.8	252,000	+82.6	140,000	−1.4	138,000
Other diatoms	14,000	+27.3	14,000	+27.3	14,000	+27.3	2,000	−81.8	11,000
Brachionus satanicus	80	−23.8	128	+21.9	110	+4.8	104	−0.9	105
Pedalion fennicum	36	−80.7	76	−59.4	340	+81.8	296	+58.3	187
Cyclops viridis	0	−100.0	16	+60.0	16	+60.0	8	−20.0	10
Diaptomus sicilis	0	−100.0	0	−100.0	0	−100.0	8	+300.0	2
Nauplii	8	−55.5	20	+11.1	40	+122.2	4	−77.7	18

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TABLE XII

PERCENT OF VARIATION FROM MEAN OF SERIES F

	Mean	Maximum	Minimum	Range
Nodularia	±22.2	+44.4	−21.9	66.3
Other Algae	±20.3	+27.2	−37.1	64.3
Chaetoceros	±42.0	+82.6	−78.3	160.9
Other Diatoms	±40.9	+27.3	−81.8	109.1
Brachionus satanicus	±12.8	+21.9	−23.8	45.7
Pedalion	±70.0	+81.8	−80.7	162.5
Cyclops	±60.0	+60.0	−100.0	160.0
Nauplii	±66.6	+122.2	−77.7	199.9

Table XIII shows the results of the analyses of series G. Here the total amount of plankton varies considerably since the variation of the different species is more or less parallel. The chemicals are quite constant. A summary of the variation of the different plankton forms is shown in Table XIV.

Series H. Since in the previous collections comparatively few crustaceans had been obtained, it was decided to collect larger samples. On October 15, 1915, four samples were therefore collected from approximately the same points as those of series C during clear and almost perfectly calm weather. Two liters of each sample were filtered thru fine bolting cloth. This allowed the diatoms and some of the algae to pass thru but retained all the crustaceans and rotifers. This was determined

TABLE XIV

PERCENT OF VARIATION FROM MEAN OF SERIES G

	Mean	Maximum	Minimum	Range
Nodularia	±26.4	+50.0	−47.4	97.4
Other Algae	±37.0	+74.0	−47.1	121.1
Chaetoceros	±50.8	+51.6	−81.3	132.9
Other Diatoms	±36.8	+57.9	−36.8	94.7
Brachionus satanicus	±34.7	+47.8	−62.5	110.3
Pedalion	±78.2	+153.7	−86.0	239.7
Cyclops	±35.1	+65.2	−43.5	108.7
Diaptomus	±50.0	+100.0	−50.0	150.0
Nauplii	±63.6	+78.2	−92.7	170.9

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TABLE XIII

DATE FOR SERIES G

(Abbreviations as in previous tables)

	Sample 2		Sample 4		Sample 6		Sample 8		
	Amt.	% var.	Amt.	% var.	Amt.	% var.	Amt.	% var.	Mean
Oxygen	6.0	+1.6	6.2	+1.6	6.2	+1.6	6.1	0.0	6.1
Free Ammonia	0.02	0.0	0.02	0.0	0.02	0.0	0.02	0.0	0.02
Albuminoid Ammonia	0.40	−20.0	0.40	−20.0	0.60	+20.0	0.60	+20.0	0.50
CO_3 ion	219	−3.9	217	−4.8	199	−12.7	277	+21.5	228
HCO_3 ion	618	−0.5	622	−0.2	645	+3.9	601	−3.2	621
Nodularia	78,000	+2.6	114,000	+50.0	40,000	−47.4	72,000	−5.3	76,000
Other Algae	104,000	−47.1	342,000	+74.0	172,000	−12.5	168,000	−14.5	196,500
Chaetoceros	102,000	−20.3	192,000	+50.0	24,000	−81.3	194,000	+51.6	128,000
Other Diatoms	30,000	+57.9	12,000	−36.8	12,000	−36.8	22,000	+15.8	19,000
Brachionus satanicus	1,652	+25.6	1,944	+47.8	493	−62.5	1,172	−10.9	1,315
Pedalion	28	−69.9	236	+153.7	13	−86.0	96	+3.2	93
Cyclops	52	−43.5	96	+4.3	67	−27.2	152	+65.2	92
Diaptomus	32	+100.0	8	−50.0	8	−50.0	16	0.0	16
Nauplii	72	−34.5	164	+49.1	8	−92.7	196	+78.2	110

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by an examination of the filtrate. No chemical analysis was made and the plants were not counted. The results are shown in Table XV.

TABLE XV

DATA FOR SERIES H

(Abbreviations as in previous tables)

	Sample 1		Sample 2		Sample 3		Sample 4		
	Amt.	% var.	Amt.	% var.	Amt.	% var.	Amt.	% var.	Mean
Depth	4.6	−10.7	5.2	+1.0	5.3	+2.9	5.5	+6.8	5.15
Temperature	9.5	.....	9.0	.....	9.0	.....	9.0	.....	.....
Moina	124	+129.6	23	−57.4	47	−13.0	22	−59.3	54
Diaptomus	0	−100.0	4	−48.4	27	+248.4	0	−100.0	7.75
Cyclops	2	−46.7	8	+113.3	3	−20.0	2	−46.7	3.75
Brachionus satanicus	600	+222.6	38	−79.6	64	−65.6	43	−76.9	186.0
Pedalion	8	−38.5	21	+61.5	6	−53.8	16	+23.1	13.0

At point 1 where the depth is the least the temperature is about one-half degree higher than at the other points. At this point, also, Brachionus satanicus and Moina, the most abundant animals, are present in great numbers. Whether this "swarm" was caused by the slight difference in depth and temperature one cannot say, but it is probably only a coincidence, since farther on, where the water was deeper, another swarm of Moina was noticed. The summary is given in Table XVI.

TABLE XVI

PERCENT OF VARIATION FROM MEAN OF SERIES H

	Mean	Maximum	Minimum	Range
Brachionus satanicus	±111.2	+222.6	−79.6	302.2
Pedalion	±44.2	+61.5	−53.8	115.3
Moina	±64.8	+129.6	−59.3	188.9
Cyclops	±56.9	+113.3	−46.7	160.0
Diaptomus	±124.2	+248.4	−100.0	348.4

The distribution of the two sexes of Moina in this series is interesting. On the whole the females are in the majority except at point 2 where the males are about twice as many. At the point immediately preceding,

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where a considerable number of Moina is found, the males are totally absent. The following is the detailed distribution:

Point	1	0	males	per	liter	124	females	per liter.
"	2	15	"	"	"	8	"	" "
"	3	5	"	"	"	42	"	" "
"	4	2	"	"	"	20	"	" "

Series I. In order to obtain still larger volumes of water a series of three samples was collected on August 17, 1916, with the plankton pump at a depth of about two and a half meters from an anchored boat. The weather was calm and when the two last samples were taken the sun went under a thin cloud, which hardly had any effect on the light. Each sample consisted of five gallons (18926 cc.) and was concentrated to 18.9 cc. All the animals in five cubic centimeters of the concentrated sample were counted and the results are shown in table XVII.

TABLE XVII

DATA FOR SERIES I

(Abbreviations as in previous tables

	Sample 1		Sample 2		Sample 3		
	Amt.	% var.	Amt.	% var.	Amt.	% var.	Mean
Asplanchna	3	+30	1	−56.5	3	+30	2.3
Pedalion	73	−12	78	−6	98	+18.1	83.0
Brachionus satanicus	54	+4	54	+4	48	− 8	52.0
Nauplii	55	+10	50	0	45	−10	50.0
Diaptomus	7	+40	3	−40	5	0	5.0
Cyclops	6	+81.8	2	−39.4	2	−39.4	3.3
Moina	0	−100	3	+30	4	+73.9	2.3

In these collections the adult crustaceans are comparatively few, but since a large volume was collected and counted the results may be considered reliable. In the case of the adult crustaceans the variation is large while in the case of the Nauplii and the rotifers it is rather small. The summary of the variation is shown in Table XVIII.

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TABLE XVIII

PERCENT OF VARIATION FROM MEAN OF SERIES I

	Mean	Maximum	Minimum	Range
Pedalion	±12	+18.1	−12	30.1
Brachionus satanicus	±5.3	+4	− 8	12.0
Nauplii	±6.7	+10	−10	20.0
Diaptomus	±26.7	+40	−40	80.0
Cyclops	±53.5	+81.8	−39.4	121.2
Moina	±68	+73.9	−100	173.9

Series J. A series of five surface samples were collected on August 27, 1916, from points lying about 100 meters apart in the center of Creel Bay as shown on the map. The lake was perfectly still and had been so for over twelve hours. The volume of each sample and the portions counted were the same as in series I. The results obtained are shown in Table XIX.

These results confirm those already obtained. Diaptomus and Moina are rather numerous, still the variation shown is large. The Nauplii and Rotifera are not as abundant as usual but their distribution is rather uniform. The temperature and the depth are almost constant, and from previous work it may be concluded that the chemicals vary but slightly. Table XX shows the summary of the variation.

TABLE XX

PERCENT OF VARIATION FROM MEAN OF SERIES J

	Mean	Maximum	Minimum	Range
Pedalion	±31.6	+48.4	−45.2	93.6
Brachionus satanicus	±31.1	+55.5	−33.3	88.8
Nauplii	±15.1	+24.5	−35.9	60.4
Diaptomus	±50.2	+112.8	−71.8	184.6
Moina	±25.1	+58.8	−21.6	80.4

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TABLE XIX

DATA FOR SERIES J

(Abbreviations as in previous tables)

	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		
	Amt.	% var.	Amt.	% var.	Amt.	% var.	Amt.	% var.	Amt.	% var.	Mean
Asplanchna	1	.....	0	.....	0	.....	0	.....	0	.....	.....
Pedalion	17	−45.2	41	+32.3	24	−22.6	28	−9.7	46	+48.4	31
Brachionus satanicus	7	−22.2	11	+22.2	6	−33.3	7	−22.2	14	+55.5	9
Nauplii	56	+5.7	66	+24.5	34	−35.9	52	−1.9	57	+7.6	53
Diaptomus	29	−25.6	11	−71.8	43	+10.2	83	+112.8	27	−30.8	39
Cyclops	1	.....	0	.....	1	.....	2	.....	0	.....	.....
Moina	81	+58.8	53	+3.9	40	−21.6	41	−19.6	40	−21.6	51

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Discussion of Results

As far as one can tell from the results the total amount of plankton seems to have a quite uniform distribution, except in a few cases where most of the organisms occur in large or small numbers in a certain place or at a certain time. No correlation is shown between the animals and the plant or chemical constituents. Without further investigation it cannot be said, however, that plankton animals are not in any way affected by the amount of phytoplankton or dissolved chemicals. The variation in the latter may be due largely to experimental error. It is probable that if there are variations in their distribution they are small and not likely to cause movements of the plankton animals. The depth and the tmeperature are always nearly constant for the whole series and the small variations that occur do not show any effect on the distribution of the plankton. All of the plankton species show an uneven distribution on all occasions, even when the individuals of a certain species are very numerous. Table XXI gives a summary of the variation of the individual species. The figures are obtained by taking the average of the mean per cent of variation from the mean and of the range of variation of all the series in which the particular species is present in numbers large enough to be considered. The table shows that the crustaceans have the least uniform horizontal distribution, the mean varia-

TABLE XXI

Mean Value and Range of "Percent of Variation from Mean" of All the Series

	No. of series averaged	Mean	Range
Nodularia	5	±29.8	93.6
Other Algae	5	±22.1	70.1
Chaetoceros	3	±42.5	127.0
Other Diatoms	3	±49.1	149.5
Brachionus satanicus	9	±49.8	148.2
Brachionus mülleri	4	±48.6	142.6
Pedalion	9	±40.7	117.1
Asplanchna	1	±57.5	218.7
Moina	4	±55.3	184.9
Cyclops	7	±48.5	136.0
Diaptomus	6	±54.6	172.8
Nauplii	8	±40.3	125.8

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tion for the adults of the three species being about ±53%. For the four species of rotifers the average is ±46%, and that of the plants is about ±30%. As has been stated before the experimental error in case of the plants is probably large, and may account for a great part, or all, of the variation found. For the rotifers and the crustaceans the experimental error is much smaller, owing to the large portion of the sample counted and to the fact that the animals are more easily retained in filtering, the variation found consequently being due almost entirely to the uneven distribution. It is not probable that the small number of individuals that are sometimes found is sufficient to explain many of the variations, since in series H, where some of the species are very numerous, the variations are above the average.[7]

Direct observations were also made by examining the water surface for aggregates of animals. During the summer of 1915, before Moina appeared, crustaceans were frequently not seen for large areas but when they occured there were usually several together. On two occasions in August and September Moinas were present in the open water a few meters from the shore, so numerous that they could be seen from a distance of several meters. These "swarms" covered an area of about one-half to one meter in diameter while the surrounding water was almost free from these animals. Also when series H was collected two aggregates of crustaceans, chiefly Moina, were noticed in the center of Creel Bay. Here, however, the areas were larger than, and not as distinct as those near the shore. During the summer of 1916, such aggregates were seen on several occasions by Dr. Young and by the writer, both near the shore and out in the open water.[8]

Comparisons of the results With Those Obtained by Other Investigators

In every case that is known to the writer the collections for the study of the horizontal distribution of the plankton have been made with a plankton net and, with but few exceptions, have concerned only the total amount of plankton. These results usually show a small variation but tell nothing of the distribution of the individual species. As far as one can tell from the data for Devils Lake the distribution of the plankton as a whole in that lake seems to be similar to the distribution elsewhere.

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A study of the subject was made on Lake St. Clair, Michigan, by Reighard in 1893.[9] He collected fourteen series, each consisting of two (in one case of three) successive hauls made in the same place. The volume of plankton per square meter of surface was then determined and the average amount in each series computed. In figuring the percentages of variation of the different catches from the average Reighard uses the volume of each catch as a basis while in this paper the average of the series is used as a basis. In order to make the results comparable the latter method has been applied to Reighard's data. For all but one series the plus and minus variations, and consequently also the mean variation, are the same since there are only two collections. Table XXII gives the plus or minus variation for each series as calculated from Reighard's data.

TABLE XXII

VARIATION PERCENTAGES OBTAINED FROM REIGHARD'S DATA

Series	II	Variation from mean in percent	±30.1
"	III	" " " " "	±17.0
"	IV	" " " " "	0.0
"	V	" " " " "	±13.9
"	VI	" " " " "	±14.6
"	VII	" " " " "	0.0
"	VIII	" " " " "	±5.4
"	IX	" " " " "	±3.1
"	X	" " " " "	0.0
"	XIII	" " " " "	±9.5
"	XIV	" " " " "	±4.3
"	XV	" " " " "	±14.7
"	XVI	" " " " "	±10.3
"	XVIII	" " " " "	±8.4
Average	value for all the series	" "	±9.4

In figuring the results by Reighard's method the variations of series II of the above series become +23.1% and −43.1%. The last variation is much greater than any other and Reighard states that it "is possibly sufficient to be referable to a 'swarm.' "

Similarly Apstein[10] studied the distribution in some of the German lakes by collecting thirty-one series, each of from two to five hauls. All the catches of a series were taken from equal depths from different parts of a lake. The catches (eighty in all) were undoubtedly all obtained from the pelagic zone at equal depths, but it is probable that the depth

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of the lake differed at the different stations. The mean value of the variation from the mean of all the series is found to be ±5.5%, corresponding to ±9.4% for Reighard's data. The highest variation found by Apstein is +22.8%.

Apstein also counted the individual species in three series, (one of three and two of two catches) and reports some data published by Zacharias in 1895. From Apstein's data I computed the percentages for Diaptomus and Cyclops of two of the series, the catches of the third being made in widely different parts of the lake. Tables XXIII and XXIV give Apstein's figures together with the percentages.

TABLE XXIII

RESULTS OBTAINED FROM APSTEIN'S DATA FOR DOBERSDORFER SEE

(Amt.=number per cubic meter. % var.=variation from the mean in percent)

	Sample 27a		Sample 27e		Sample 27c		
	Amt.	% var.	Amt.	% var.	Amt.	% var.	Mean
Cyclops	122,088	+6.7	93,024	−18.6	128,016	+11.9	114,377
Diaptomus	328,320	−7.6	198,208	−44.5	539,947	+51.8	355,492

TABLE XXIV

RESULTS OBTAINED FROM APSTEIN'S DATA FOR GR. PLÖNER SEE

(% var.=percent of variation from mean)

	No. pr. catch	% var.	No. pr. catch	% var.	Mean
Cyclops	887	−1.5	915	+1.5	901
Diaptomus	26	−13.3	34	+13.3	30
Nauplii	372	+13.1	286	−13.1	329

In Dobersdorfer See Cyclops shows a mean variation of ± 12.4% and a range of 30.5%. For Diaptomus the figures are: ±34.5% and 96.0%. In the case of Gr. Plöner See the variation for each catch and the mean variation are the same since there are only two series. The percentages are much lower than those for Dobersdorfer See.

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In the series collected by Zacharias Hyalodaphnia has a mean variation of ±7% and a range of 15.7%, and the copepods a mean of ±5.9% and a range of 15.1%. (Table XXV). These figures, as well as those of Apstein, are much lower than the Devils Lake figures, and correspond more nearly with those obtained by Reighard and by Apstein for the total amount of plankton.

TABLE XXV

RESULTS OBTAINED FROM ZACHARIAS' DATA

Amt. = number per volume. % var. = variation from mean)

	Schlossgarten		Alesborg		Rott's Gart'n		
	Amt.	% var.	Amt.	% var.	Amt.	% var.	Mean
Hyalodaphnia	630	+10.5	540	−5.2	540	−5.2	570
Copepoda	720	−8.9	840	+6.£	810	+2.5	790
Bosmina	150	0.0	150	0.0	150	0.0	150

Kofoid[11] tested the longitudinal distribution of the plankton in Illinois River by making "a series of ten catches in immediate succession from a boat anchored in mid-channel." The current was flowing at the rate of nearly two miles an hour, and the time required for collecting was about two hours. The catches therefore represent plankton taken from a body of water about three miles in length. From the centrifuged material the volume of plankton per cubic meter was computed and the following percentages were obtained: mean ±3.6%, maximum +8.6%, minimum −5.5%, and the range 14.1%. Kofoid's results thus show a smaller variation than those of Reighard and of Apstein, but cannot very well be compared to samples obtained from a lake, especially during calm weather.

The distribution of Daphnia hyalina in Lake Geneva was studied by Gandolfi-Hornyold and Almeroth[12] during the summer of 1913. Vertical hauls were made with a net and the number of individuals in each catch were counted. All the catches taken from the same depth on the same day were then compared, regardless of the location and the depth of the lake at the place where the collections were made. From some data given by the authors the percentages were computed and are shown in Table XXVI.

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TABLE XXVI

RESULTS OBTAINED FROM DATA GIVEN IN GANDOLFI-HORNYOLD AND ALMEROTH'S TABELLE I

Depth of Lake in m.	Depth of catch in m.	Number per catch	Mean	% var. from mean	Mean var. in %	Range of var. in %
40 E 45 E 15 E 10.5 E 17 E	10-0	E 3 E E 9 E E 41 E E 7 E E 61 E	24	E −87.5 E E −62.5 E E +70.8 E E −70.8 E E+154.2 E	±89.2	241.7
45 E E 40 E	20-0	E 185 E E E E 276 E	230.5	E −19.7 E E E E +19.7 E	±19.7	39.4
40 E E 40 E	30-0	E 236 E E E E 333 E	284.5	E −17.0E E E E +17.0 E	±17.0	34.0

The catches taken by hauling the net from a depth of ten meters show a large variation, but the percentages decrease as the depth and the number of organisms increase. In some cases the depth of the lake varies considerably but this does not seem to have any effect on the number of organisms.

"Swarms" or aggregates, similar to those seen in Devils Lake, have been discussed several times by different investigators, e.g. by Huitfeldt-Kaas,[13] Reighard,[14] and Ward.[15] The aggregates usually consist of Cladocera and in many cases they have been observed near the shore, but occasionally in free water. No great consideration is given them, however, since they are supposed to occur but seldom and therefore do not greatly effect the results of quantitative plankton studies. The comparatively irregular distribution shown by the zooplankton in Devils Lake is in all probability very constant since about the same results were obtained for all the series. The methods used are quite thoro and no large error is possible. It is significant, also, that both the Sedgwick-Rafter method and the pump method give about the same variation percentages in many cases. It is hardly conceivable that the organisms in Devils Lake should have a more irregular distribution than those elsewhere, but no work has been done that can be exactly compared to that

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done on Devils Lake. Gandolfi-Hornyold and Almeroth's results show a large variation of Daphnia hyalina but the distances between the collecting stations were probably great and there were differences in depth and probably also in temperature. Apstein's and Zacharias' counts of the individual species show a distribution quite similar to that usually found for the total amount of plankton.[16] Catches made with a net, as in the above mentioned cases, represent the number of individuals in a vertical column of water, and it is possible that the vertical distribution for the different catches differed altho the total amount for the entire column differed but slightly from that of another column. If this were the case the horizontal distribution for the different levels would differ. The collections made in Devils Lake to test the vertical distribution (see table I) show a large difference in the number of animals of a column of water between two periods of collecting. Moreover when a large volume of water is collected, especially with a net, the differences in distribution tend to be reduced, since by this method several thickly populated portions of water may be included, while on the other hand the small sample usually collected for filtration in the Sedgwick-Rafter method may be obtained entirely either from a volume of water containing a "swarm" or from one where the organisms are scarce. This method is consequently the more precise for studying the local distribution of plankton forms.

Nor can the distribution of the total amount of plankton in Devils Lake be compared to that in other lakes, since the data for the former concern the individual species only. In the majority of cases it appears, however, that some species make up in volume or weight for the difficiency caused by others. Since the main portion of plankton usually consists of algae a large variation of the animals does not greatly effect the distribution of the plankton as a whole.

Conclusions

From the results obtained by the study of the horizontal distribution of the plankton in Devils Lake the following conclusions may be drawn:

(1) The zooplankton in Devils Lake shows a great irregularity in horizontal distribution, and this irregularity cannot be correlated with any variations in amount of phytoplankton or in the chemical and physical environment. It is more likely due to the habit of swarming among plankton animals, due perhaps to a social instinct, similar to that found

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in many other groups of the animal kingdom. Plankton swarms are at times visible, even at considerable distances, to the naked eye.

(2) With larger samples (19 litres) the variations tend to be reduced, but even here they are at times greater than in the smaller ones (½ litre).

(3) Similar, tho in general smaller variations have been found by other workers, but no exact comparison with their results is possible, since their methods have been different.

(4) Definite conclusions regarding the distribution of the phytoplankton can not be drawn, owing to the inaccuracy in the method of its emuneration. In general, uowever, it appears to be more uniformly distributed than the zooplankton.

(5) These variations invalidate the usual assumption that a given sample of water is representative of a large area, at least in respect to its animal inhabitants, and necessitate the collection of large numbers of samples before definite conclusions regarding their distribution or movement can be drawn.

NOTES

[1] Owing to Mr. Moberg's absence on military duty in France, I have taken the liberty of editing his paper, adding some observations as footnotes and making a few changes in the text. The conclusions are mainly my own, but apart form these, and a few other minor alterations, the paper is his. R. T. Young.

[2] See Whipple "The Microscopy of Drinking Water." 1914, pp. 28 et seq.

[3] The chemical analyses were made by Dr. Fred H. Heath of the University of North Dakota.

[4] Whipple, *l.c.* p. 42.

Whipple, *l.c.* p. 41.

[6] In the Journal of the Quekett Microscopical Club, Vol. XI, pp. 373-4, Rousselet has described a new species of Brachionus from Devils Lake, under the name of spatiosus. As this form closely resembles B. mülleri, and numerous transitional forms occur, it is here included in the latter species. R. T. Y.

[7] In series H, only two litres of water were taken. In series I and J, in which 19 litres were taken, the variations are seen in general to be smaller than the average, as is to be expected. (Compare tables XVIII and XX and XXI) Even here, however, some of the variations exceed the average, while others are almost as great. (Compare the range of 184.6 for Diaptomus in series J, table XX, with the average for this genus of 172.8 in table XXI; Cyclops 121.2 in series I, table XVIII, with the average, 136.9 in table XXI, and Moina, 173.9 in series I with the average of 184.9 in table XXI.) In general, the more numerous the individuals of a species, the smaller the variations in their number. This also is to be expected. The variations in the phytoplankton

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are probably partly attributable, as Mr. Moberg has stated, to experimental error. In part they are probably also due to chance variations in distribution. For example, in one case in which Nodularia was exceedingly abundant, I observed it clumped together in numerous small patches. If one or two of such masses happened to be included in a 500 cc sample, while another sample was free from them, they would readily explain the observed differences. Many of the variations in the zooplankton may also probably be due to chance, especially in those series where only 500 cc of water were filtered. Even so they indicate the difficulty, if not impossibility of obtaining reliable results by the Sedgwick-Rafter method, in the case at least of the zooplankton.

Such an assumption is, however, wholly inadequate to explain such a variation as is shown by Brachionus satanicus in samples 3 and 4, ser. D. table VII, in one of which 380 individuals were present in 500 cc, while another contained 0. Similarly 124 Pedalion were present in one of these samples and none in another. Vice versa, sample 3, in which no rotifers whatever occurred, contained 12 Cyclops, while sample 4, in which rotifers were abundant contained only 4 Cyclops. The comparatively few Cyclops present can hardly have determined the difference in number of the rotifers. The two samples were taken at points only about 200 metres apart in the main body of the lake which is roughly 15 x 7 Km in extent. The day was clear with but little wind and the physical and chemical conditions at the two stations were virtually identical, as may be seen in table VII. An explanation of such variations, as due either to chance or experimental error is, in my opinion, wholly excluded.

For further evidence of a similar character see Diaptomus, samples 2 and 4, ser. J, table XIX, in which 19 litres were sampled; Moina and Brachionus satanicus, samples 1 and 2 and Diaptomus, samples 3 and 4, ser. H, table XV, in which two litres were sampled; Brachionus Satanicus and Nauplii samples 3 and 4, Ser. E, table IX; Pedalion, samples 1 and 5 and Nauplii, samples 5 and 7, Ser. F, table XI, and Brachionus Satanicus, and Pedalion, samples 4 and 6, and Nauplii, samples 4, 6 and 8, Ser. G, table XIII.

These conclusions are furthermore supported by direct field observations. (See f.n. 8) R. T. Y.

[8] The following is from my notebook: "9–17–17. I notice copepod swarms very clearly today. In places, usually in streaks, the water is milky with Diaptomus, in others very few. Occurred at surface. Sunny . . . 9–18–17. I notice numerous copepod (mostly Moina) swarms in the surface water near shore, these forming streaks in the water visible plainly at a distance of several feet. I made a collection of one of these swarms, 500 cc, which I concentrated by filtering thru No. 20 bolting cloth. Collection made by simply dipping up some of the swarm in a quart jar. . . "

This collection when concentrated to 30 cc and counted gave approximately 70,000 individuals per litre! This number, moreover, is probably somewhat too low, owing to a number of the animals adhering to the pipette in transferring to the counting cell. R. T. Y.

[9] Reighard "A Biological Examination of Lake St. Clair," Bulletin of the Michigan Fish Commission, 1894, No. 4.

[10] Apstein "Das Süsswasserplankton, Methode and Resultate der quantitative Untersuchung," 1896, pp. 51 et seq.

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[11] Kofoid "The Plankton of the Illinois River," Bulletin of the Illinois State Laboratory of Natural History, 1903, pp. 269 et seq.

[12] Gandolfi-Hornyold and Almeroth, "Mitteilungen über die Verteilung von Daphnia hyalina Leydig im Genfer See (Petit Lac), Internat. Revue d. ges. Hydrobiol. u. Hydrogr., 1915, Bd VII, pp. 426-432.

[13] Huitfeldt-Kaas, "Plankton in Norwegischen Binnenseen," Biol. Centralblatt, 1898, Bd XVIII, pp. 625 et seq.

[14] Reighard, *l.c.* p. 32 et Seg.

[15] Ward "A Biological Examination of Lake Michigan" Bulletin of the Michigan Fish Commission, 1896, No. 6, pp. 62-64.

[16] In the case of Cyclops and Diaptomus for Dobersdorfer See, Apstein finds a somewhat larger variation. The mean for Cyclops is 12.4%, and for Diaptomus 34.5%. For Diaptomus the maximum is +51.8% and the minimum —44.2%, making a range of 96%. In the 10-0 meter catches Gandolfi-Hornyold and Almeroth find a large variation, but in the catches from greater depths it is considerably smaller.

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DEPARTMENT OF NOTES AND REVIEWS

It is the purpose, in this department, to present from time to time brief original notes, both of methods of work and of results, by members of the Society. All members are invited to submit such items. In addition to these there will be given a few brief abstracts of recent work of more general interest to students and teachers. There will be no attempt to make these abstracts exhaustive. They will illustrate progress without attempting to define it, and will thus give to the teacher current illustrations, and to the isolated student suggestions of suitable fields of investigation.—[Editor.]

GENETICS IN RELATION TO AGRICULTURE

Under this title Professors Babcock and Clausen have brought together in a most valuable way two winning groups of interests. The development of agriculture as an application of various underlying sciences has been one of the very creditable outcomes of scientific progress. And the growth of the educational aspects of agriculture has been the wonder of modern education, which with the aid of shrewdly used political appeals has made more than one of our universities the tail to an agricultural kite. On the other hand, none of the divisions of biological science approaches that of genetics in the impetus which it has given in recent years to research. This is true whether we are thinking primarily of the discovery of new facts or of the theoretical conclusions to be had from them. If therefore we acquiesce, as we probably must, in the authors' statement that no field of science contributes more of economic worth than genetics does to the complex called agriculture, we have a measure of the possibilities of a book on this subject. In the opinion of the reviewer the book is peculiarly valuable, not merely to agricultural students for whom it is primarily written, but for teachers and students of biology everywhere, for the general reader, and for the breeder. A very rich selection of illustrative material has been made—much of it from sources not familiar to the general student.

The subject is treated under three heads: Fundamentals; Plant Breeding; Animal Breeding.

Part 1, dealing with the Fundamentals of genetics comprises fourteen chapters. Biologists will agree, I believe, that the various hypotheses have been fairly stated and the pros and cons of the more uncertain questions justly given. The illustrative material is pertinent and modern.

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Part 2 discusses Plant Breeding in twelve chapters and contains such representative chapters as, Historical Introduction, Varieties in Plants, Composition of Plant Populations, Selection, Utilization of Hybrids, Mutations, Graft Hybrids and other Chimeras, Breeding Plants for Disease Resistance, Methods of Plant Breeding.

Part 3, Animal Breeding includes thirteen chapters. These run parallel to those of part 2, with some of peculiar interest added—as for example, Disease and Related Phenomena in Animal Breeding, Sex Determination in Animals, Fertility in Animals, and Some Beliefs of Practical Breeders. The latter deals briefly with the scientific grounds for disbelief in telegony, maternal impression, prepotency, and the like.

The concluding chapter states the grounds for a becoming modesty in relation both to the quantity and the precision of our present knowledge of animal genetics.

The book contains also a glossary, a list of the literature cited, and an adequate index. It is richly illustrated with pictures, diagrams, and tables. It is an attractively made book, and is sure to prove a useful and satisfying one.

GENETICS IN RELATION TO AGRICULTURE, by E. B. Babcock and R. E. Clausen. Pp. xx+675, fully illustrated. The McGraw-Hill Book Company, New York, 1918.

NITRATE CELLULOSE AS A SUBSTITUTE FOR CELLOIDIN

As a result of the war the importation of celloidin has been interrupted and the microscopist has been compelled to look about for workable, substitutes. Parlodion has been found to be very satisfactory, and can be obtained from the Arthur H. Thomas Company, Philadelphia. In this laboratory, however, we have had such excellent results with nitrate cellulose (soluble cotton) that I feel justified in calling it to the attention of other workers. Although never in very general use, soluble cotton as an embedding medium has been known for some time, and has been used for a number of years in the laboratory of Dr. Adolf Meyer, John Hopkins Hospital, as a routine method of embedding. It has two valuable features—the cost is less than any of the other practical celloidin substitutes, and its preparation is comparatively simple.

Nitrate cellulose is shipped in strong alcohol, and upon reaching the laboratory is put through the following process: It is washed first in several changes of 95% alcohol and squeezed nearly dry; then in two changes of absolute alcohol, after which it is dissolved in equal parts of absolute alcohol and ether, filtered through absorbent gauze into a flat

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dish and placed under a bell jar to evaporate until dry. It is then cut into thin strips and put into a thermostat for several hours at a temperature of 37, the door of the thermostate being left ajar to allow for the escape of the ether fumes. When the chips are thoroughly dry they are stored in air-tight bottles ready for use. Where haste is necessary the filtration through gauze may be dispensed with, the cotton being decanted as it dissolves and evaporates slowly under a bell jar. The bottles are then placed in the thermostat under the same conditions as described above. This, however, is a crude method, useful in ordinary work, but not to be followed where careful infiltration is desired.

For embedding we use the same technique as for celloidin. Eight wide mouthed, cork-stoppered bottles are cleansed and *thoroughly dried*. The solutions are made up in such a way that each 100 cc. contains 2, 4, 6, etc., up to 16 grammes (by weight) of the soluble cotton. Tissue that has been thoroughly dehydrated and immersed in equal parts of absolute alcohol and ether, is then passed through these graded solutions, being left 24 hours in each. If the tissue is to be cut immediately it is mounted on a fibre block and hardened in chloroform or in 80% alcohol.

Nitrate cellulose can be obtained from Maas & Waldstein, New York.

CHAS. H. MILLER

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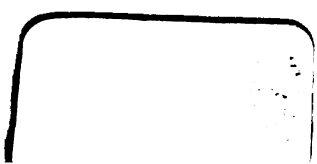

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